COMPARATIVE BIOLOGY OF TWO FORMS OF AN INVASIVE VINE, *DOLICHANDRA UNGUIS-CATI* (L.) LOHMANN (BIGNONIACEAE): IMPLICATIONS FOR WEED SPREAD AND BIOCONTROL

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A thesis submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy

2016

Keywords

Anatomy, Bignoniaceae, biodiversity, biological control agents, biomass accumulation, cat's claw creeper, competitiveness, colonization, disturbance, ecological strategies, ecophysiology, efficacy, epidermis, fitness traits, fluctuating resource hypothesis, foliar nectaries, functional traits, intraspecific variation, invasive species, invasiveness, invasion ecology, leaf economic spectrum, long pod, *Macfadyena unguis-cati*, morphology, palisade mesophyll, performance, phenotypic integration, phenotypic plasticity, physiology, photosynthetic rate, plant-herbivore interactions, plant invasion, plant sexual reproduction, polyembryony, propagule pressure, relative growth rate, resource use efficiency, seed ecology, seed germination, short pod, SEM, SLA, successful colonizers, taxonomy, trait correlation, tubers, weed management, WoNS, woody vine.

Thesis Abstract

Cat's claw creeper, *Dolichandra unguis-cati* (Bignoniaceae) is a Weed of National Significance (WoNS) in Australia and a major environmental weed in Queensland and New South Wales states. Two forms of this weed ('short pod' and 'long pod') occur in Australia. Short pod is widely distributed in Australia, but long pod is only found in a few localities in southeast Queensland. There is a general lack of understanding why the two forms are not equally prevalent. Previous studies have shown significant differences in the flowering phenology and leaf morphology of the two forms. Despite these differences, the same biological control agents are used in the management strategies for the two forms. Preference tests have not been performed to determine whether biological control agents would choose one form over the other.

The aims of this study were twofold, firstly, to use a trait-based framework to compare germination, anatomical and physiological traits between long pod and short pod. This aim included an assessment of trait responses to different water, light and nutrient resource conditions. Secondly, the study sought to test the preference of two biological control agents, *Carvalhotingis visenda* and *Hylaeogena jureceki* for the two forms under different water and nutrient resource conditions.

The study found short pod to have significantly higher germination rates and higher levels of polyembryony than long pod. Short pod also exhibited significantly higher germination plasticity than long pod. Short pod foliar anatomy indicated presence of thicker leaves and significantly higher frequency of foliar nectaries than long pod. Short pod had a less compact spongy mesophyll with larger intercellular spaces than long pod. Only one type of epidermal hair (unicellular trichomes) was observed in short pod. Conversely, long pod had two types of epidermal hairs (unicellular and multicellular trichomes). The distribution of unicellular trichomes was higher in long pod than in short pod.

Short pod performed better than long pod, as indicated by production of higher biomass and more tubers and branches under low nutrient resources. Short pod exhibited higher values of carbon assimilation, water use efficiency and leaf nitrogen than long pod in response to water, light and nutrient resources. However, long pod produced more biomass than short pod under high light and nutrient resource conditions. Phenotypic integration did not differ between long pod and short pod when considering all resource levels. However, short pod exhibited significantly higher

phenotypic integration when high light and nutrients were considered separately. Short pod developed a significantly higher number of tubers than long pod in response to water, light and nutrient resources. Overall short pod performed better than long pod in response to different resource conditions. A multivariate exploration of functional traits using principal components analysis showed a clear separation of the two forms along the second axis. The second axis was influenced by shoot/root ratio, tuber development, WUE, leaf nitrogen and quantum yield of photosystem II. Biological control preference results show that *C. visenda* does not have a preference for any form while *H. jureceki* have a preference for long pod over short pod. Resource level had a significant effect on preference for both forms, with agents choosing the high nutrient plants the most.

Results from this study make a significant contribution to our understanding of why short pod is the prevalent form in Australia. Short pod was shown to exhibit more traits that are associated with fast growing invaders. Higher rates of germination and polyembryony could have contributed to the spread of short pod. Higher values for relative growth rates, tuber biomass and branching shown by short pod are traits that enhance colonization success. Moreover, short pod exhibited higher phenotypic integration and greater germination plasticity in response to different levels of resources than long pod, indicating greater capacity to invade environmentally heterogenous habitats. Although long pod did not perform as well as short pod for most traits, accumulation of higher biomass under high light and nutrient conditions imply potential for colonization success by this form under disturbance scenarios.

Lack of preference for either form by *C. visenda* implies that this agent is suitable for continual use against long pod and short pod. On the contrary, preference for long pod by *H. jureceki* implies a potential lack of efficacy of this agent on the more prevalent short pod form. A preference pattern by agents in the field could jeopardize biological control efforts for *D. unguis-cati* in Australia. More research needs to be carried out in the field to substantiate findings from this study. An evaluation of biological control method against *D. unguis-cati* is suggested, especially in light of occurrence of long pod and short pod.

The striking differences in life history traits between long pod and short pod in this study inevitably raise questions about the taxonomy of the two forms. Differences in germination traits and frequency of polyembryony, growth patterns, tuber development and response to environmental conditions have taxonomic implications for the two forms. Differences in leaf anatomical traits such as types of hairs and nectaries have taxonomic implications for the two

forms, and have previously been used in taxonomic resolution in Bignoniaceae. Thus, the outcomes of this study corroborate a previous hypothesis that these two forms may be different species. A phylogenetic analysis of the genus *Dolichandra* with extensive sampling of all possible forms of *D. unguis-cati* from both the native and introduced range is recommended. To test the biological species concept, we recommend studies that will test whether the two forms of *D. unguis-cati* can interbreed. As there were significantly higher germination rates and greater polyembryony in the short pod than long pod, flower or seed-eating biological control agents would be appropriate in the management of the short pod.

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Table I Abbreviations and descriptions of traits measured in this study and their units

Variable	Description	Units
Anatomical traits		
Epidermal thickness	Thickness of both the adaxial and abaxial epidermis	µm
Palisade/Spongy mesophyll thickness	Thickness of the palisade/spongy mesophyll layer	µm
EFN	Number of foliar nectaries per area	mm^{-2}
SD	Number of stomata per area	mm^{-2}
Trichomes density	Number of trichomes (hairs) per area	mm^{-2}
Germination traits		
GRI	Germination rate index	
T_1	Time it takes to initiation of germination	Weeks
T_{50}	Time it takes to 50% germination	Weeks
T_{65}	Time it takes to 65% germination	Weeks
Physiological traits		
A_{max}	Area based photosynthetic rate	µmol m^{-2} s^{-1}
A_{mass}	Mass based photosynthetic rate	µmol g^{-1} s^{-1}
E	Transpiration rate	mol m^{-2} s^{-1}
WUE	Water use efficiency	µmol CO_2mol^{-1} H_2O
PNUE	Photosynthetic nitrogen use efficiency	µmol mol s^{-1}
RUE	Resource use efficiency	
N_{area}	Area based leaf nitrogen concentration	g m^{-2}
N_{mass}	Mass based leaf nitrogen concentration	mg g^{-1}
Chl.	Chlorophyll content	SPAD units
ϕPSII	Apparent quantum yield of photosystem II	(ΔF/Fm’
C	Leaf carbon concentration	g m^{-2}
C:N	Carbon nitrogen ratio	
Performance traits		
Biomass	Total dry mass	g/plant
SRR	Shoot root ratio	
BSD	Stem diameter at the root-stem junction	mm
ADI	Apical dominance index (number of secondary branches divided by length of the branch)	m^{-1}
SMR	Shoot mass ratio	
TRR	Tuber root ratio	
Tuber biomass	Total tuber dry mass	g/plant
LA	Leaf area	cm^2
LAR	Leaf area ratio	
SLA	Specific leaf area	cm^2 g^{-1}
LDMC	Leaf dry matter content	mg g^{-1}
LMA	Leaf matter per area	
RGR	Relative growth rate	mg g^{-1} $month^{-1}$

Table II Additional abbreviations of words and their meanings used in this thesis

Abbreviations	Meaning or description
LP	'Long pod' form of *D. unguis-cati*
SP	'Short pod' form of *D. unguis-cati*
PAR	Photosynthetically active radiation, µmol photon m^{-2} s^{-1}
ANOVA	Analysis of variance
MANOVA	Multivariate analysis of variance
PCA	Principal component analysis
SEM	Scanning Electron Microscope
SE	Standard error of the mean
LES	Leaf economic spectrum
QUT	Queensland University of Technology
DAF	Department of Agriculture and Fisheries
QLD	Queensland
SEQ	Southeast Queensland
WoNS	Weeds of National Significance
HLHN	High Light x High Nutrients condition
HLLN	High Light x Low Nutrients condition
LLHN	Low Light x High Nutrients condition
LLLN	Low Light x Low Nutrients condition
HWHN	High Water x High Nutrients condition
HWLN	High Water x Low Nutrients condition
LWHN	Low Water x High Nutrients condition
LWLN	Low Water x Low Nutrients condition

Table III Definition of key terms used that will be used throughout this Thesis

Term	Definition	References
Invasive species	Naturalised non-native plant species that produce viable offspring in large numbers and spreading to novel habitats at a fast rate, in the process causing negative impacts on the environment.	(Colautti and MacIsaac 2004; Kolar and Lodge 2001; Lee 2002; Pyšek and Richardson 2010)
Plant invasion	The process where introduced plants spread to novel areas away from introduction sites	(Levine 2000; Lodge 1993; Vitousek *et al.* 1996; Williamson 1996)
Colonization	Spread and encroachment of a species into a community, and continual reproduction in the new environment	(Bazzaz 1986; Crawford and Whitney 2010; Pannell 2015)
Native species	Plant species that originate and occur in a given geographical area, having arrived without intentional/unintentional human agency. Native plants are synonymous with indigenous species.	(Daehler 2003; Pyšek *et al.* 2004; Reichmann *et al.* 2016; Thompson *et al.* 1995)
Non-native species	Plant species that occur in an area that they are not native to. In some invasion literature, non-native plants are synonymous with exotic, introduced plants or non-indigenous species	(Keane and Crawley 2002; Müller-Schärer and Steinger 2004; Pyšek *et al.* 2004; van Kleunen *et al.* 2010b)
Functional traits	Anatomical, morphological, physiological, reproductive or phenological characteristics of plants that reflect plant fitness or performance	(Adler *et al.* 2014; Drenovsky *et al.* 2012; Kunstler *et al.* 2016; Pérez-Harguindeguy *et al.* 2013)
Disturbance	Any event that disrupts vegetation assemblages and changes resource availability e.g. fires, nutrient addition, forest clearing/canopy removal, flooding etc.	(Davis *et al.* 2000; Dechoum *et al.* 2015a; Firn *et al.* 2008; Hobbs and Huenneke 1992; Lonsdale 1999; Mack and D'Antonio 1998)
Phenotypic plasticity	The ability of a genotype to adjust its phenotype in response to biotic and abiotic environmental conditions	(Agrawal 2001; Bradshaw 1965; Lande 2015; Nicotra *et al.* 2010; Pigliucci 2001; Schlichting 1986; Sultan 2000)
Phenotypic integration	Pattern of functional covariance among different plant traits or joint variation of two traits in response to environmental change	(Luo *et al.* 2015; Osunkoya *et al.* 2010b; Osunkoya *et al.* 2014; Pigliucci 2003; Schlichting and Pigliucci 1998)
Polyembryony	A germination phenomenon where a multiple seedlings emerge from a single seed.	(Blanchard *et al.* 2010; Mendes-Rodrigues *et al.* 2012; Oka *et al.* 2016; Salomão and Allem 2001; Webber 1940)

Statement of Original Authorship

The work contained in this thesis has not been previously submitted to meet requirements for an award at this or any other higher education institution. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made.

QUT Verified Signature

Signature:

Date: _____25/11/2016_____

Acknowledgements

Many thanks go to my principal supervisor Dr. Tanya Scharaschkin for accepting me as her student and for believing in me when I articulated from a Masters to a PhD Programme. That means a lot to me and for that I really am grateful. Your style of supervision has taught me to pay attention to little details, especially in scientific writing. And thanks for caring, you always asked about my family and thanks for that cosy little blanket you made for my baby when she was born. That was priceless. I thank Associate Professor Jennifer Firn, an internal associate supervisor and a continual source of inspiration. You inspired me to learn R and your knowledge of ecology has always amazed me. I learnt from you to always be positive and optimistic and ask questions. You always had answers and recommendations when I was confused. Thanks for believing in my abilities even in moments when I didn't. Thanks to the two most amazing external supervisors I was privileged to have. Thanks to Dr. Dhileepan for your contagious laughter and friendship, from you I learnt that life goes on even after a Journal has rejected a manuscript. You were always willing to help and listen. And thanks a lot for your patience and together with Tanya for organising a scholarship for me from DAF. Gratitude is an understatement in this regard. Regarding scholarships, sincere thanks also go to Jenn and Tanya for the persistence you demonstrated to secure the Supervisor scholarship for the last six months of my candidature. Thanks a lot to Dr. Segun Osunkoya for your friendship and the long discussions we always had about life in general and everything in between. Thanks for personal advice you have given to me on life and career. Those long skype, WhatsApp and phone calls we had will always be engraved in my memory because I learnt a lot of things as a result. To my whole supervisory team, thank you for your continued support, commenting and correcting my thesis and manuscript drafts. I will forever be grateful for things I have learnt in the process.

Sincere thanks go to my best friend and lovely wife Kakale 'Kay' Buru for resigning from your job to be with me during this PhD programme. Thanks for your love and encouragement and for always asking "How are things going?" a question that was always not very easy to answer I must confess! Thanks for enduring my absence without complaining, especially during major milestones of the PhD. I love you to the moon and back, this PhD is as much yours as is mine! Thanks to the cutest and most amazing angels I am blessed to call my daughters, Agy (6) and Litzy (3) for being the inspiration for me to keep on keeping on! Daddy loves you so much!

I sincerely acknowledge my mom, Gladys Chikadzi Chakalisa and mom-in-law Ikageleng Motlhabani for all your motherly care and concern for me and my family, despite our being thousands and thousands of kilometres away from home. I am especially indebted to my late grandma (who I referred to as mom!) – Lumbo Buru for being a huge part of my childhood life and for the discipline you instilled in me, I loved you dearly and always wanted to be successful in life so that I can take care of you!

Thanks to the Plant Structure & Systematics Group and the Jenn Firn Ecology Group (both at QUT) for reading drafts and for great companionship. Thanks to all the friends I have made in Brisbane and the Gold Coast during my PhD days. Thanks to Ps Michael for your well wishes and prayers. I would like to specially thank Prof. Baleseng Moseki for being the first to introduce me to research during my undergraduate degree at the University of Botswana. Thanks to all the great teachers that have inspired me along the journey of my life as a student.

I acknowledge technical support from EEBS technicians at QUT - Mark Crase, Amy Carmichael, Anne-Marie McKinnon, Sharyn Berg and Karina Pyle. I acknowledge CARF technicians - Hellen O'Connor, Sanjleena Singh and Rachel Hancock for assistance, training and advice in processing leaf samples, embedding, sectioning and image capture acquisition. I would like to thank Erica Porter for helping with collection of stomatal density data. Cristina Latorre is acknowledged for all the SEM work presented in this study (Chapter 3).

Thanks a lot to Sarie Gould, Noelene Davis, Courtney Innes (EEBS) and Tiziana LaMendola (SEF) for always being so friendly and very helpful with financial and administrative issues. Thanks to Dr. Marion Bateson and Dr. Cheryl Desha for agreeing to be in my internal examination panel and for their valuable comments.

I wish to gratefully acknowledge sources of funding for my PhD and conference travels as follows:

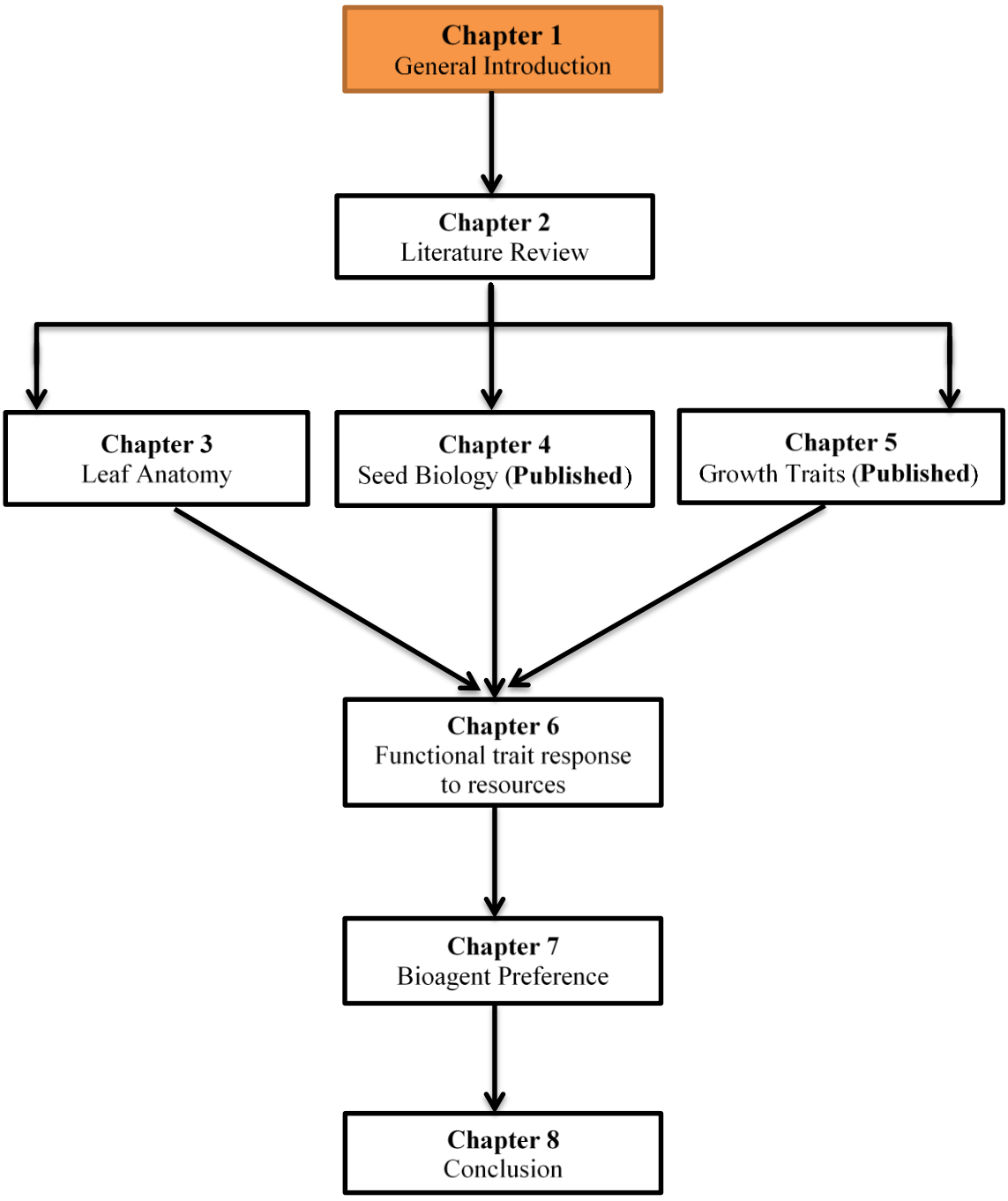
1) Government of Botswana for paying tuition and stipend for the first two years of my PhD programme.

2) Department of Agriculture and Fisheries, Biosecurity Queensland for a scholarship that paid my stipend during my third year. Thanks too for paying for Glasshouse facilities for my experiments and for buying all the supplies I needed for my research.

3) QUT: For a tuition waiver scholarship for the last one and half years of my PhD. Thanks to QUT, Earth Environment and Biological Sciences (EEBS) for the Supervisor Scholarship that paid my stipend for the last six months of my PhD.

4) Australasian Systematic Botany Society (ASBS), Weed Society of Queensland (WSQ) and Council of Australasian Weed Societies (CAWS) for student travel awards to travel to conferences.

Above all, I would like to thank God for his grace during my PhD. Faith in God kept me positive, calm and optimistic during moments when the going was getting tough.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Chapter 1: General Introduction

1.1 Background

A key aspect of invasion ecology is to understand properties of introduced species that enhance their invasive capabilities to colonize novel environments (Reichmann *et al.* 2016; Richardson and Pyšek 2006). Trait-based comparative frameworks involving introduced species and native ones have been used to achieve this (Bradley *et al.* 2010; Daehler 2003; Finerty *et al.* 2016; Hui *et al.* 2016). Often ecologists choose certain easy or "soft" traits that are directly linked to "hard" fitness and performance traits and compare them between invasive and non-invasive species (Funk *et al.* 2016). Some reproductive, dispersal, physiological and morphological traits have consistently shown a clear dichotomy between invasive and non-invasive species (Adler *et al.* 2014; Luo *et al.* 2015; Rejmanek and Richardson 1996; Williamson and Fitter 1996a).

Life history traits that enhance propagule pressure such as production of numerous small seeds that are easily dispersed and high germination plasticity have been associated with invasive species (Colautti *et al.* 2006; Lloret *et al.* 2005; Lockwood *et al.* 2009; Simberloff 2009; Sun *et al.* 2012; Wainwright and Cleland 2013). Higher values of performance traits such as specific leaf area (SLA), biomass and relative growth strategies (RGR) are frequently associated with invasive species (Feng *et al.* 2007; Osunkoya *et al.* 2010a; Pattison *et al.* 1998b; Poorter and Remkes 1990; Reich *et al.* 1998; Wilson *et al.* 1999). Invasive species show higher phenotypic plasticity than non-invasive ones, a trait that enables them to successfully colonize environmentally heterogenous habitats (Agrawal 2001; Firn *et al.* 2012; Geng *et al.* 2016; Minden and Gorschlüter 2016) but see Dostál *et al.* (2016).

Although trait based studies have undoubtedly advanced the state of knowledge of how functional traits drive plant community assemblages, at times such studies reach contradicting conclusions (Oduor *et al.* 2016; Palacio-López and Gianoli 2011). For example, several pairwise studies have found that both invasive and non-invasive species have similar traits under the same environmental conditions (Jo *et al.* 2016; Meiners 2007; Palacio-López and Gianoli 2011). Leishman *et al.* (2010) observed that invasive and non-invasive species have similar carbon capturing strategies (also see Parker *et al.* 2013). A recent review of this 'holy grail' of plant ecological strategies by Funk *et al.* (2016) further reveals that significant trait differences are sometimes detectable even within a species. Parker *et al.* (2013) argue that

while there may be differences between some invasive and non-invasive species, this pattern is not universal. In line with this, Lemoine *et al.* (2016) posit that invasive and non-invasive species use the same strategies of becoming abundant, stressing that comparisons of introduced against native species could yield a false dichotomy. In light of the conflicting outcomes of trait-based pairwise studies, Li *et al.* (2016) suggest a comparison of closely related taxa such as hybrids, varieties or subspecies. Comparisons between non-native species that have multiple forms or varieties occupying similar habitats but exhibiting different levels of invasion success could yield more informative outcomes (Kolar and Lodge 2001). Some invasive species that have variable forms include *Lantana camara, Acacia nilotica* and *Dolichandra unguis-cati* (Shortus and Dhileepan 2011; Urban *et al.* 2011; Wardill *et al.* 2005).

Cat's claw creeper, *Dolichandra unguis-cati* (L.) Lohmann (syn. *Macfadyena unguis-cati* (L.) Gentry) in Australia is an appropriate system for trait-based comparisons. This species was introduced into Australia for ornamental purposes from South America in the 1800s (Dhileepan 2012; Downey and Turnbull 2007; Gentry 1976). *Dolichandra unguis-cati* is now a major invasive species in Australia and considered as a Weed of National Significance (WoNS) (Thorp and Lynch 2000). Two forms of *D. unguis-cati* with significantly different abundance levels occur in Australia (Shortus and Dhileepan 2011). The short pod (SP) form occurs extensively in Queensland and New South Wales, often in dense assemblages, while the long pod (LP) form occurs in a few localities of southeast Queensland (Dhileepan 2012). The cause of the variation in abundance and prevalence between the two forms is not known, but different ecophysiological performances and variable responses to resources could be a potential reason.

The major aim of the study presented in this thesis was to compare functional traits of the two forms of *D. unguis-cati* so as to explain the differences in their prevalence. This was achieved by comparing a range of performance and fitness traits between LP and SP under similar conditions and in response to variable resource conditions. From a weed management perspective, preference tests of two biological control agents were conducted on LP and SP. The preference tests were necessitated by the fact that whereas there are morphological and prevalence variations between LP and SP, the same biological control agents are used to control both forms (Dhileepan *et al.* 2007a; Dhileepan *et al.* 2013; Dhileepan *et al.* 2010). Prior to this study, evaluation of biological control agents and their efficacy against the two forms of *D. unguis-cati* had not been carried out.

1.2 Study Objectives

There are five main objectives of this study, which are as follows:

1. To determine if there are differences in foliar anatomical traits between LP and SP. The study is performed to create a baseline data of leaf anatomical traits, especially those that could have taxonomic, ecophysiological and biological control implications. A previous study by Osunkoya *et al.* (2014) found some significant differences in certain anatomical traits between SP and a native congener, *Pandorea jasminoides*.

2. To compare the seed biology and germination dynamics of LP and SP in response to different levels of light and temperature regimes. Germination is an important stage in the life history of *D. unguis-cati* that facilitates its spread into new ranges. A seed ecology study by Vivian-Smith and Panetta (2004b) showed that SP germination requirements were non-specific and observed multiple seedlings emerging from single seeds, but the germination pattern for LP remains unknown.

3. To compare leaf and whole plant traits such as SLA, number of tubers and growth rates of LP and SP grown under similar conditions over time. In a field experiment by Taylor and Dhileepan (2012), using plants generated from tubers, LP was found to have higher relative growth rate than SP. In this study we used similar aged plants generated from seeds to determine growth patterns of the two forms.

4. To assess physiological and performance-related traits and their responses to different levels of light, water, and nutrient resource conditions. This is the first study to compare physiological performance of LP and SP in response to resource conditions.

5. To determine whether two biological control agents, a leaf sucking tingid (*Carvalhotingis visenda*) and a leaf mining beetle (*Hylaeogena jureceki*) show any differences in preference for either LP or SP under different resource conditions.

1.3 Thesis outline

The thesis is divided into the literature review (Chapter 2) and chapters that address each of the objectives outlined above. The five experimental chapters (3-7) are presented as stand-alone research articles, each with a separate introduction, materials and method, results and discussion. Chapter 8 links all the chapters together, summarises and discusses the main findings of the study and gives suggestions for future research directions.

Chapter 1: General Introduction

Chapter 1 is the present chapter and it outlines general objectives of the study and gives general background to the thesis. This chapter also gives a brief introduction of all the other chapters of this thesis.

Chapter 2: Literature Review

This chapter reviews relevant literature on invasiveness traits to provide theoretical context of the questions asked and justification of the choice of methods from similar studies. A review of *D. unguis-cati* literature is presented with a focus on what is currently known about the two forms and what that means for invasiveness and weed management.

Chapter 3: Leaf Anatomy

This chapter describes and compares the basic leaf anatomy and micro-morphological characters of LP and SP, with a special focus on those traits that could potentially have ecophysiological and performance implications. Anatomical traits are also discussed in relation to their implication for biological control and taxonomy.

Chapter 4: Seed Biology

This chapter describes and discusses germinability, germination rates and occurrence of polyembryony of LP and SP in response to different light and temperature regimes. Light and temperature regimes used are similar to prevailing conditions in habitats where the two forms occur. The seed biology results are discussed in light of their implications for potential spread of the two forms of *D. unguis-cati* in Australia. Chapter 4 has been published in an open access journal, American Journal of Plant Sciences (AJPS) (doi: 10.4236/ajps.2016.73058) and can be accessed at http://www.scirp.org/Journal/PaperInformation.aspx?PaperID=65100. The first page of this paper is included in appendix A of this thesis.

Chapter 5: Growth and Performance Traits

This chapter outlines growth rates and biomass allocation patterns of LP and SP plants generated from seeds and grown in low nutrient resources. Functional traits in this chapter are discussed in the context of their implications on enhancing invasiveness potentials of LP and SP. This chapter has also been published in an open access journal, NeoBiota (doi: 10.3897/neobiota.30.8495) and can be accessed at http://neobiota.pensoft.net/articles.php?id=8495. The first page has been included in appendix B of this thesis.

Chapter 6: Physiological and Performance Traits Response to Resources

This chapter describes and discusses the response of eco-physiological and performance traits to two levels of light and water resources, both factored with two levels of nutrients. High light and high nutrient conditions are reminiscent of disturbance regimes that are known to facilitate colonization by invasive species.

Chapter 7: Preference of Biological Control Agents

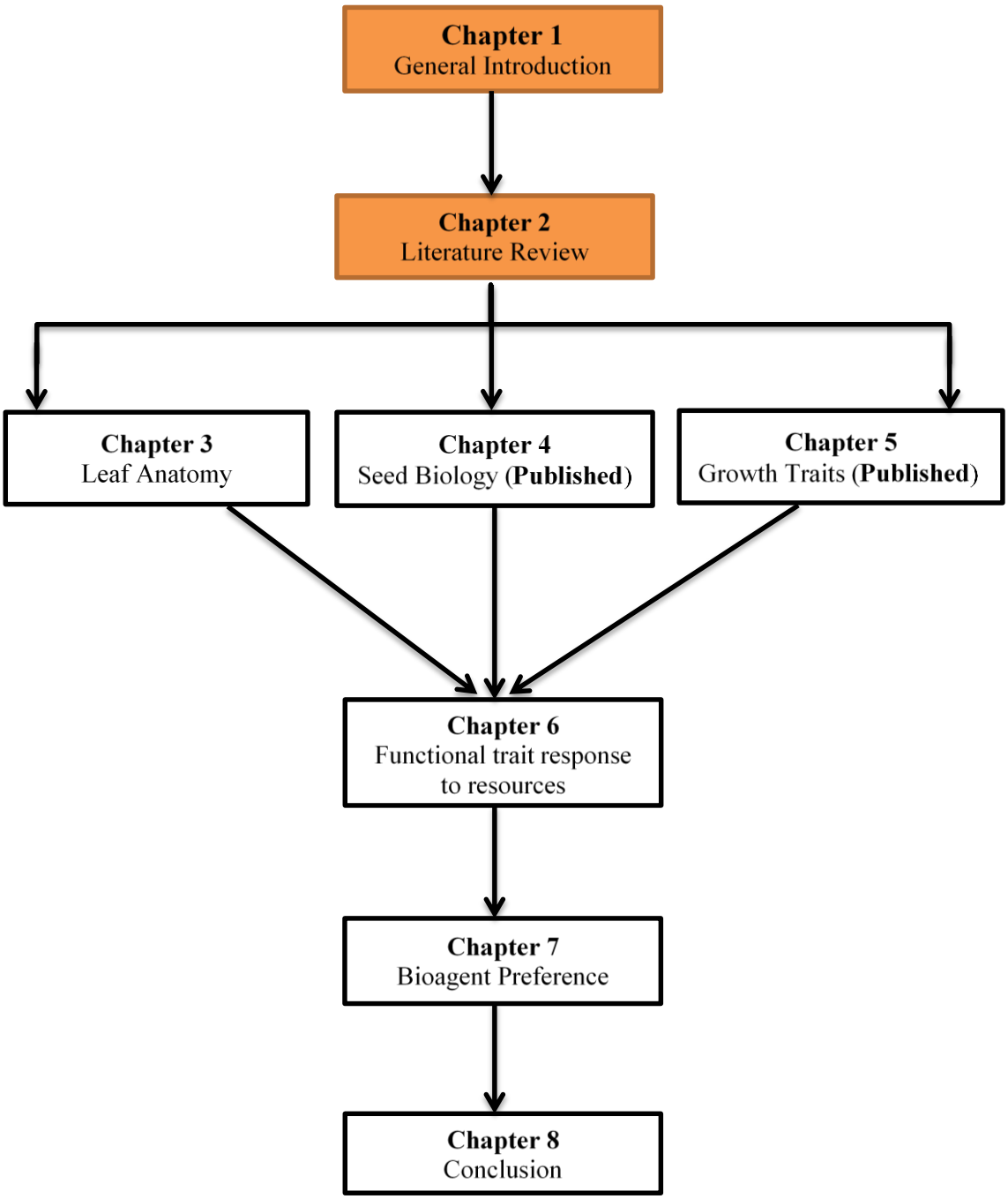
This chapter describes the preference patterns of the two biological control agents tested on both LP and SP under control, water and nutrient treatments. Oviposition and leaf damage caused by biological control agents are discussed in light of their implications for efficacy of biological control programmes for LP and SP.

Chapter 8: Conclusion and Future Directions

This chapter links together findings from the entire study and attempts to address the issue of different levels of prevalence between the two forms. This is achieved by way of extrapolating implications of the study findings to invasiveness, weed management and taxonomy. Questions arising from the study are presented as suggestions for future research on *D. unguis-cati*.

Notes on Thesis Preparation

Chapters 3-7 of this thesis describe and discuss individual and independent experiments that each has a discrete set of objectives, methods and discussion sections particular to each experiment. As a result, there is some necessary repetition of information that may already be in the literature review (Chapter 2) so that there is coherence in each chapter. This is particularly true for Chapters 4 and 5 that have already been published. On the other hand, the introduction sections of Chapters 3, 6 and 7 that have not been published will not repeat most of the background information already included in Chapter 2. Figures and Tables are embedded within the text. At the beginning of every chapter is a 'thesis flow diagram' that indicates the particular chapter highlighted in orange.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Chapter 2: Literature Review

Dolichandra unguis-cati in Australia: A tale of two species?

2.1 Abstract

Cat's claw creeper, *Dolichandra unguis-cati* (L.) Lohmann (syn. *Macfadyena unguis-cati* (L.) Gentry) is a major environmental weed in Australia. Two forms of this weed ('long pod' and 'short pod') occur in Australia. The long pod form occurs in a few localities in southeast Queensland, while the short pod form is widely distributed in Queensland and New South Wales. Recent studies have shown that these two forms have distinctive leaf morphology and fruits that differ in size and number of seeds. As the two forms have significantly different abundance levels in Australia, they could have different success rates of invasiveness which would warrant different management strategies. The morpho-anatomical differences between the two forms may compromise the efficacy of various management options, including biological control strategies. It has been hypothesised that significant variation in a weed in the novel range could potentially impede the effectiveness of biological control agents. Thus, this review aims to consolidate current literature on the two forms of *D. unguis-cati* with the view to identify research gaps and prioritise future lines of research. The review places the study aims and methods into theoretical perspective, justifying the choice of functional traits measured in this study.

2.2 Introduction

Cat's claw creeper, *Dolichandra unguis-cati* (L.) Lohmann (syn. *Macfadyena unguis-cati*) is a perennial vigorous woody climbing vine in the angiosperm family Bignoniaceae (Gentry 1973). Its leaves are simple or compound, opposite, dark green on the adaxial (upper) surface and a lighter green on the abaxial (lower) surface (Downey and Turnbull 2007).

Compound leaves are trifoliate with the terminal leaflet often modified into a tough, trifid, claw-like tendril that grips objects and surfaces during climbing (Gentry 1983). The claw-like tendril is the feature from which this species common name, cat's claw creeper, is derived (Gentry 1983) *Dolichandra unguis-cati* regenerates sexually through the production of numerous papery seeds and asexually (vegetatively) through production of subterranean tubers (Downey and Turnbull 2007; Osunkoya *et al.* 2009).

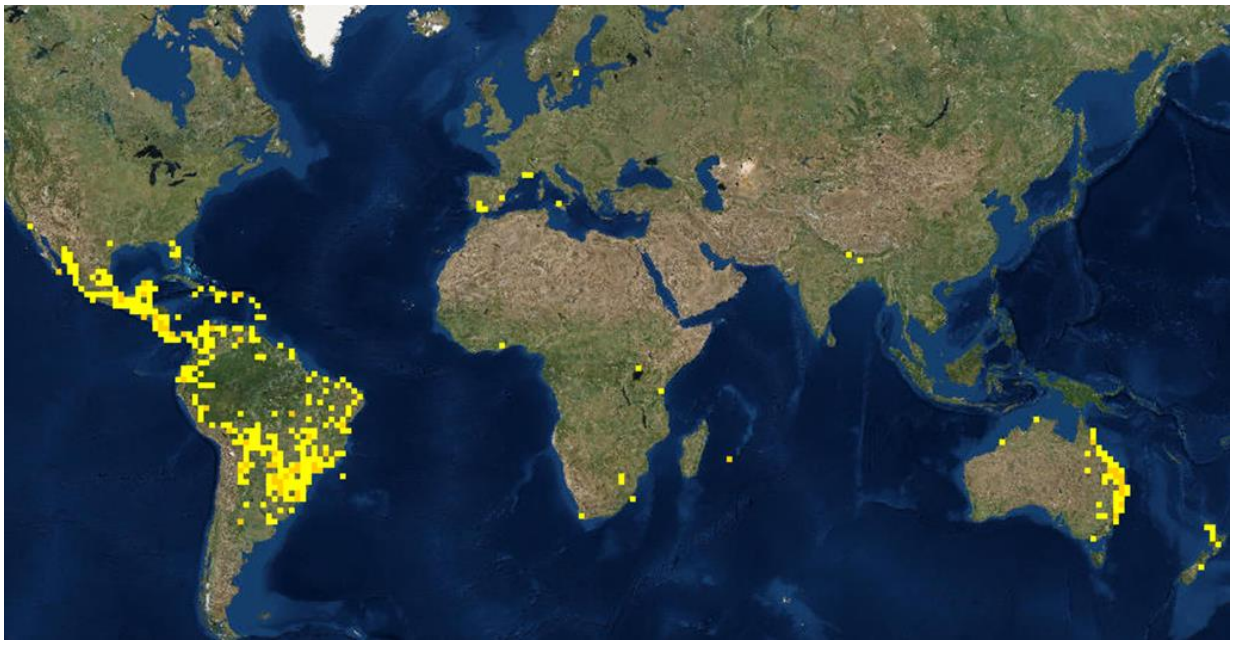


Figure 2.1. Global distribution of *D. unguis-cati* according to the Global Biodiversity Information Facility (GBIF).

Dolichandra unguis-cati is native to the islands of the Caribbean, Mexico, Central America and South America to Argentina (Gentry 1983; Howard 1989; Lohmann 2006; Lohmann and Taylor 2014) **(Figure 2.1)**. It was introduced to Australia as an ornamental plant because of its bright and showy yellow flowers and climbing habit (Dhileepan 2012; Downey and Turnbull 2007) . The first record of this species in Australia appeared in a Melbourne nursery catalogue in 1865 and later reported to be naturalised in Queensland by the 1950s (Downey and Turnbull 2007). Today, this species is regarded as a major environmental weed that threatens indigenous vegetation in coastal and sub-coastal areas of Queensland and New South Wales (Batianoff and Butler 2003; Dhileepan 2012; Downey and Turnbull 2007). *Dolichandra unguis-cati* has recently been listed as one of the Weeds of National Significance (WoNS) in Australia (Dhileepan *et al.* 2013) because of its damage to the environment and great potential for further spread (http://www.weeds.org.au/WoNS/). Being listed as a WoNS

means that *D. unguis-cati* has had detrimental consequences, both economic and environmental, at a landscape and regional scale and hence requires national strategies to reduce its impact. It also means that it is prioritised for control under the National Weeds Strategy (Thorp and Lynch 2000). The Weeds of National Significance Strategic Plan provides a basis for a coordinated management of the WoNS by setting out objectives and corresponding control actions (Australian Weeds Committee 2013). As a WoNS, this species is prohibited for sale in all the territories and states of the Commonwealth of Australia.

In the state of Queensland, *D. unguis-cati* is considered a Class 3 Weed under the Land Protection (Pest and Stock Route Management) Act of 2002. Under this act, a Class 3 weed is one that is already established and having adverse economic and environmental impacts (Land Protection Act 2002). In the state of New South Wales (NSW) it is a Class 4 weed under the Noxious Weeds Act of 1993 (Treviño *et al.* 2006). Under this act, a Class 4 weed poses a threat to the environment and has potential to spread further. In the state of South Australia, although not expected to naturalise due to prevailing cold weather conditions (also see Buru *et al.* 2014), this species is prohibited from sale and movement in any form under the Natural Resources Management Act of 2004 (http://www.pir.sa.gov.au/__data/ assets/pdf_file/0018/223632). In Western Australia, it is on the prohibited invasive species list and is not allowed entry into the state. Only one specimen of this species is lodged at the Western Australia Herbarium (specimen PERTH 708761) and it was collected from the town of Broome (http://biocache.ala.org.au/occurrences/ce7daea0-2869-4eff-8473-75518c3cb6d2).

Apart from Australia, *D. unguis-cati* has also been introduced to other parts of the world and has naturalised on all the continents except Antarctica (Starr and Starr 2008) **(Figure 2.1)**. It is considered an environmental weed in many countries of the world such as South Africa, Uganda, Zimbabwe (Dhileepan 2012; Sparks 1999; Williams 2002), Egypt (Aboutabl *et al.* 2008), China (Huang *et al.* 2009; Liu *et al.* 2006), Niue (Space and Flynn 2000), New Caledonia (Meyer 2000), Hawaiian Islands and Florida in the USA (Ewers *et al.* 1990; Francis 2004; Morgan and Overholt 2005; Wong 2007), New Zealand (King and Dhileepan 2009; Sykes 1981) and some parts of Europe (de Almeida and Freitas 2006; Gassó *et al.* 2010; Prentis *et al.* 2009). As a result, it is listed in the Global Invasive Species Database (GISD) (De Poorter and Browne 2005).

Dolichandra unguis-cati presents a serious threat to native biodiversity, especially in riparian and rainforest plant communities (Dhileepan 2012; Downey and Turnbull 2007). As a

liana, *D. unguis-cati* is regarded as a structural parasite with the ability to transform ecosystems (Ewers *et al.* 2015; Raghu *et al.* 2006; Stevens 1987). Where there is standing vegetation, it smothers tree canopies, and the biomass can build to a point where it causes the collapse of canopy structures (Batianoff and Butler 2003) **(Figure 2.2a)**. In the absence of vertical support, it readily grows along the ground, forming dense mats that preclude recruitment, growth and germination of indigenous understory vegetation (Downey and Turnbull 2007; King *et al.* 2011a) **(Figure 2.2b)**. This growth pattern transforms natural habitats into monospecific stands, resulting in loss of floral biodiversity and changes in soil biota and physico-chemical properties (Osunkoya *et al.* 2011; Perrett *et al.* 2012).

Figure 2.2. *Dolichandra unguis-cati* infestations in Queensland, Australia: a) Vertical growth smothering over trees; b) Thick mats of intertwining horizontal stems creeping on the ground. These pictures were taken in Oxley where LP and SP co-occur.

2.3 The increased problem of *D. unguis-cati* in Australia

To complicate the status of *D. unguis-cati* as a weed in Australia, two morphologically and phenologically distinct forms of this species occur in Queensland (Shortus and Dhileepan 2011; Sigg *et al.* 2006; Taylor and Dhileepan 2012). The forms of *D. unguis-cati* have been informally named long pod (LP) and short pod (SP) due to differences in their average fruit

length at maturity (**Figure 2.3**). (LP: 700.2 ± 23.5 mm; SP: 300.9 ± 89.6 mm) (Shortus and Dhileepan 2011). The fruits are capsules but have been informally referred to as pods. LP and SP are known to carry an average of 120 ± 10 and 60 ± 23 seeds per pod at maturity, respectively (Shortus and Dhileepan 2011). Seeds of both forms are two-winged, papery and flattened/oblong in shape, 10 - 18 mm long, 4.2 – 5.8 mm wide. The average seed biomass is not significantly different between the forms (mean seed biomass for LP: 16.60 ± 0.65 mg and for SP: 15.65 ± 0.83 mg) (Shortus and Dhileepan 2011).

Figure 2.3 Differences in mature fruits or pod length between LP (a) and SP (b) forms of *D. unguis-cati*.

Both forms have a showy yellow, trumpet-shaped flower but LP flowers have a deeper hue of yellow than SP flowers (Shortus and Dhileepan 2011). Reproductive phenology differs between the two forms: the fruits of SP mature in late summer to early autumn (February – May) whilst those of LP mature in late winter to early spring (July – September). These two forms appear to prefer similar habitats **(Figure 2.4)** and have similar growth habit **(Figure 2.5)**, although there is general lack of research on the ecology of this species (Osunkoya *et al.* 2009).

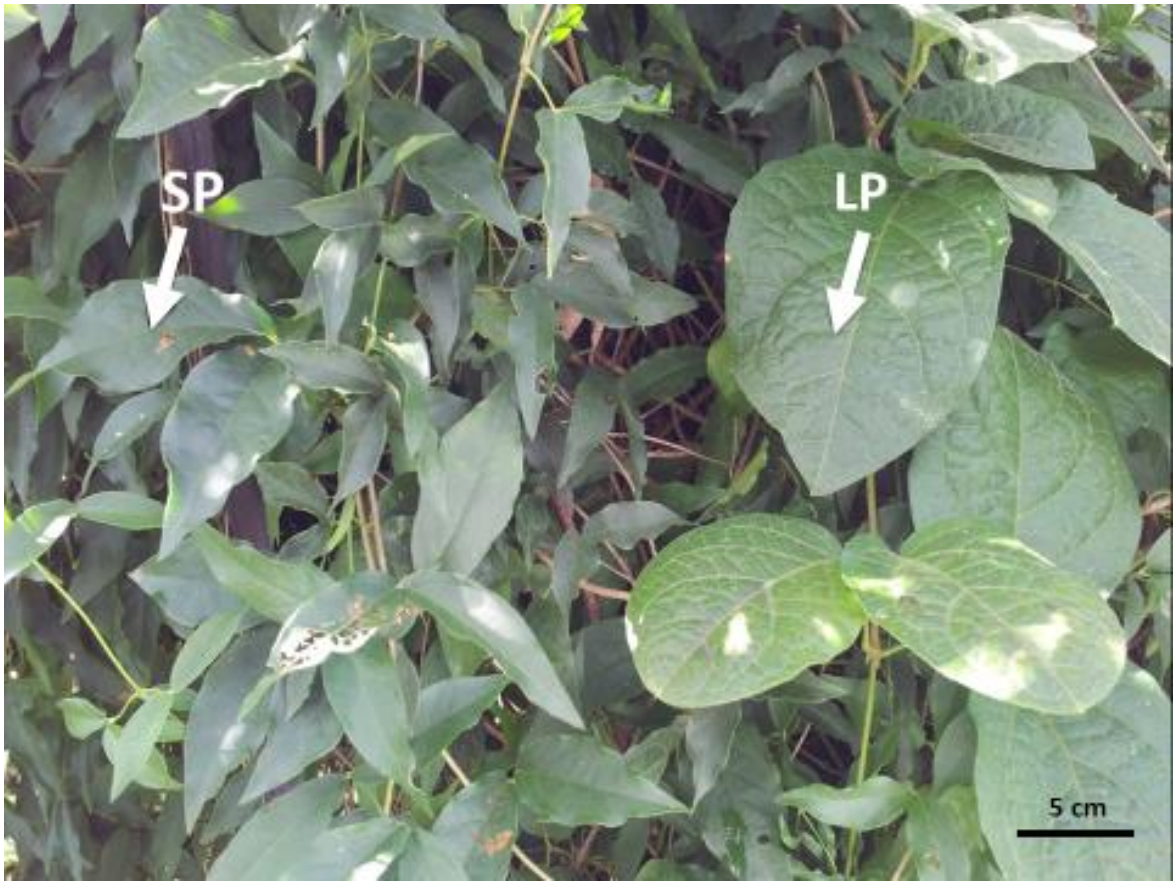


Figure 2.4. Two forms (LP and SP) of *D. unguis-cati* co-occurring and showing differences in leaf morphology.

Figure 2.5. Growth habit of the two forms (LP and SP) of *D. unguis-cati*. a: LP in flower of a fence; b: SP in flower growing along a fence.

The two forms of *D. unguis-cati* have different levels of prevalence in Australia. SP occurs extensively in Queensland and New South Wales (Dhileepan 2012; Downey and Turnbull 2007), often in dense infestations. In contrast, LP is known to occur in about 15 isolated localities of southeast Queensland, and in less dense infestations (Liz Snow, Biosecurity Queensland, pers. comm. 7/03/2016) **(Figure 2.6)**. SP appears to be the form of *D. unguis-cati* that is regarded as an environmental weed in different parts of the world (Dhileepan 2012; Prentis *et al.* 2009). LP does not appear to be as invasive as SP as it occurs in only a few localities in Australia. The cause for the observed differences in abundance levels is not yet established. However, the occurrence of distinct forms of an invasive species that exhibit variable prevalence presents a unique system that can be used to test colonization success hypotheses. Plant traits that are associated with colonization success can be measured and their differences assessed between the forms – an approach synonymous with non-native invasive vs. non-native non-invasive comparison (Gallagher *et al.* 2015).

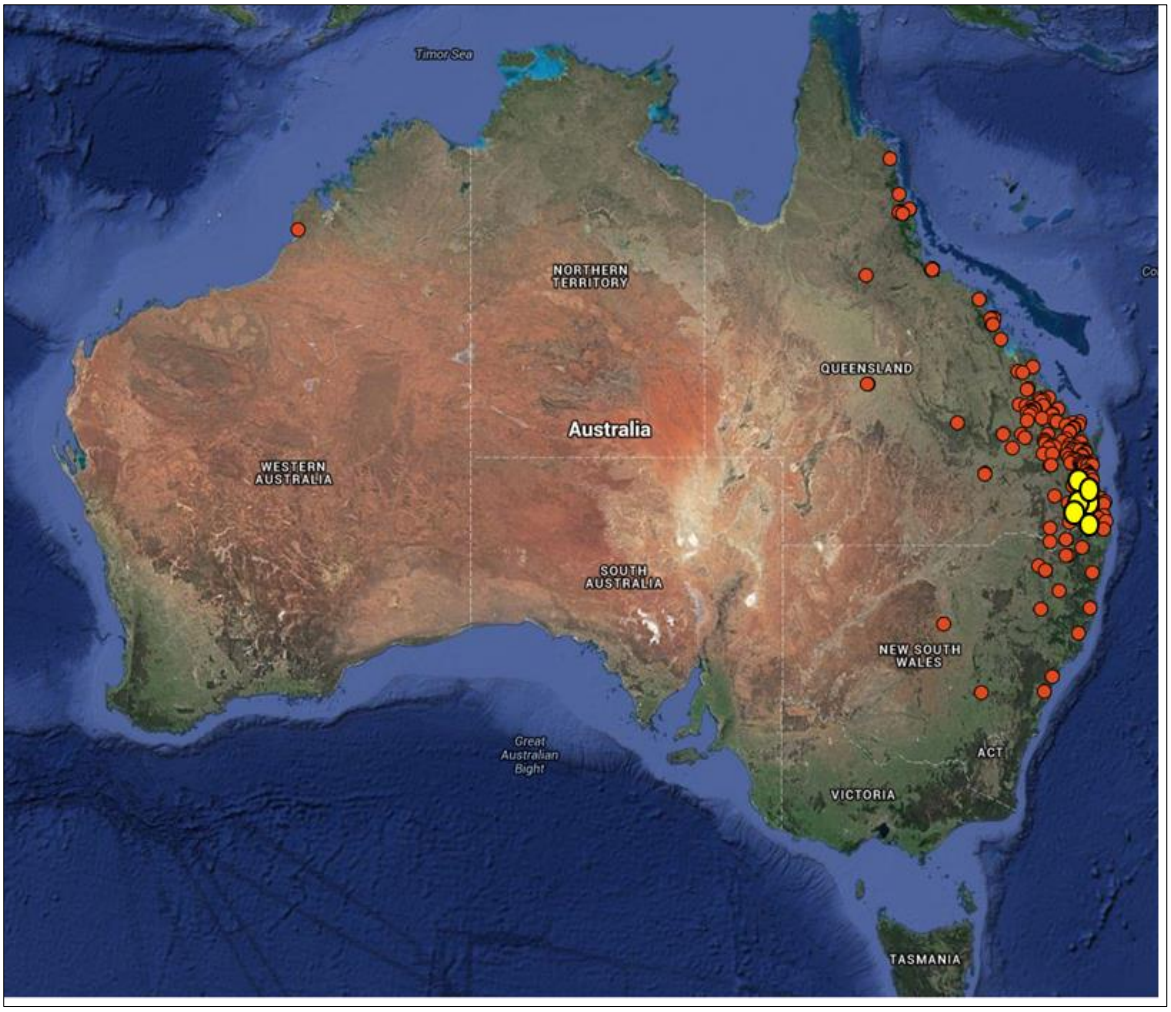


Figure 2.6. Distribution of *D. unguis-cati* in Australia. Red circles represent SP while yellow circles represent the LP.

In Australia, the management of *D. unguis-cati* takes an integrated approach which involves the use of herbicides, mechanical and biological control (Dhileepan 2012; Osunkoya and Perrett 2014). However, because of the sensitive nature of the riparian ecosystems, biological control is prioritised as the viable approach for its management in Australia (Dhileepan 2012). Biological control is the introduction of natural herbivores or pathogens from the native range of an invasive species to control it in its introduced range (McFadyen 1998; Müller-Schärer and Schaffner 2008).

In Australia, the biological control programme for *D. unguis-cati* began in 2001 (Dhileepan *et al.* 2005). The programme started with surveys for biocontrol agents in the native range of this species, especially Brazil, Argentina, Paraguay, Venezuela and Trinidad, where nine insects were identified as potential biological control agents (Dhileepan *et al.* 2005; Sparks 1999). Only three of these biological control agents have been approved for release in Australia after host-specificity tests (Dhileepan *et al.* 2007a; Dhileepan *et al.* 2013; Dhileepan *et al.* 2007b). Although these biological control agents have established in the field, it is not known whether they have been successful in reducing the spread of *D. unguis-cati*. The management of this weedy species is made more difficult by its growth pattern which includes development of dense networks of subterranean tubers (King *et al.* 2011a; Osunkoya *et al.* 2009).

The existence of these two forms may also compromise the efficacy of the biological control program of *D. unguis-cati* in Australia. This is because some biological agents are only able to survive and exert maximum damage on some forms/cultivars of a variable species (Cilliers and Neser 1991; Zalucki et al. 2007). This underscores the need to investigate whether the biological control agents currently used against this species are equally effective for both forms.

2.4 Aims and Objectives of the Literature Review

The study described in this thesis partly aimed to understand the biology and eco-physiological traits that could explain the differences in prevalence between the two forms of

D. unguis-cati. The other aspect of the study focused on the efficacy of biological control agents on the two forms. In order to place the study into theoretical perspective, this Chapter reviews the relevant literature that deal with colonization success and biological control hypotheses. Hypotheses will include (i) the enemy release hypothesis (ERH), (ii) the evolution of increased competitiveness ability hypothesis (EICA) and (iii) the ideal weed (IW) hypothesis. Appropriate literature will be reviewed to support the choice of functional traits measured in this comparative study. This is aimed at identifying proximate drivers of the variability between the two forms of *D. unguis-cati*. This review also consolidates current literature on *D. unguis-cati*, primarily from the perspective of occurrence of the two forms, and by extension the potential efficacy of management strategies.

2.5 Why are some plant species more invasive than others?

A fundamental objective of invasion ecology is to identify a suite of functional plant traits that may determine invasion success in novel environments (Pyšek and Richardson 2007; Richardson and Pyšek 2006; van Kleunen *et al.* 2010b). Comparative studies between exotic invasive species and their native non-invasive congeners (van Kleunen *et al.* 2011) and meta-analytical approaches (Pyšek and Richardson 2007; van Kleunen *et al.* 2010b), have contributed immensely to our understanding of traits that promote colonisation and invasion success. However, it has also proven difficult to consistently find a correlation of the same set of traits with invasiveness because of the varying effects of environmental factors on different plant species (Alpert *et al.* 2000; Burns 2006; Funk 2013). Studies have shown that it is not any one particular trait that confer invasiveness on all species, rather it is how a species responds to different environmental conditions that contributes to its fitness and abundance (Firn *et al.* 2012; Osunkoya *et al.* 2010a; Osunkoya *et al.* 2010b; Pattison *et al.* 1998a). Plastic responses of invasive plants to varying environmental conditions increase their competitiveness and fitness (Claridge and Franklin 2002a).

As a result of the attention that invasion ecology has received in the last few decades, several hypotheses have been proposed to explain why some plants are able to successfully

colonize novel communities (Alpert *et al.* 2000; Crooks *et al.* 1999; Levine and D'Antonio 1999; Mack *et al.* 2000; Rejmanek and Richardson 1996; Williamson and Fitter 1996b). The mechanisms of invasion described by these hypotheses are not mutually exclusive but synergistically related (Lau and Schultheis 2015). Success in colonization cannot be explained by any single hypothesis in isolation (Gurevitch *et al.* 2011). Studies have shown that during invasion there is an interaction between the susceptibility of recipient habitats and traits of the invading species to make it possible for invaders to thrive (Smith and Knapp 2001).

Generally, plant invasion hypotheses are either based on species life history traits that promote colonization success (invasiveness) or susceptibility of habitats to invasion (invasibility) (Lamarque *et al.* 2011; Williamson 1996; Williamson and Fitter 1996b). The fluctuating resource hypothesis proposed by Davis *et al.* (2000) suggests that habitats become more susceptible to colonization by invasive species when there is increase in unused resources. Any process that disturbs the equilibrium of habitats, resulting in fluctuations in resources (e.g. nutrients, water or light), opens a gap that invasive species could exploit (Dawson *et al.* 2015a; Funk and Vitousek 2007; Hobbs and Huenneke 1992; Leinaas *et al.* 2015). The following section of the literature review will discuss three main invasiveness hypotheses that are relevant to this study. The review of the ideal weed hypothesis will lead into the literature on invasiveness related plant traits, with particular emphasis on those functional traits that were investigated in this study.

2.5.1 The Enemy Release Hypothesis (ERH)

The Enemy Release Hypothesis (ERH) stipulates that when introduced to a new place, a plant species is free from both specialist herbivores and other natural enemies (Keane and Crawley 2002). The ERH assumes that in the new environment, there is greater impact of generalist enemies on the native vegetation than introduced species because of the novelty of the newcomer. This is expected to give an advantage to the introduced species over native species (Müller-Schärer *et al.* 2004). As a result of the reduction in regulation by herbivores, the introduced species experience rapid and unchecked increase in abundance.

The use of natural enemies obtained from the native range of an invasive species for its control in its introduced range is predicated on the ERH (Culliney 2005; Keane and Crawley 2002). This is called biological control of invasive species (DeBach 1991; Müller-Schärer and

Schaffner 2008). Support for the ERH hypothesis largely comes from the success rate of previous biological control programs (Keane and Crawley 2002). Biological control is a well-established method of weed management that has previously recorded significant success rates (e.g. Julien 1987). McFadyen (1998) presented a comprehensive review of biological control successes and problems associated with such programs. More recently, other successful stories of biological control programs have been reported (e.g. Fowler *et al.* 2000; Goolsby *et al.* 2016; McFadyen 2000; Palmer *et al.* 2010; Seastedt 2015), in spite of associated challenges (Waage *et al.* 2002). Classical biological control strategies use specialist (or host specific) natural enemies from the native range of the invasive plant with the aim of reducing the abundance of the invasive plant to ecologically acceptable thresholds (Culliney 2005; Müller-Schärer and Schaffner 2008). The effectiveness of a biocontrol agent lies in its ability to complete all or part of its life cycle in the target host plant, thus exerting maximum damage. Therefore, generalist herbivores may not be effective as biological control agents when compared with specialist herbivores (DeBach and Rosen 1991; Snyder and Ives 2003). However, even specialist herbivores sometimes face challenges when they are released to control a species in the introduced range due to a number of factors.

The Insect Performance Hypothesis proposed by Larsson (1989) suggests that the performance of insects increases with plant stress. Hsiao (1973) has extensively documented the impact of water deficits on different physiological and anatomical processes of plants which may affect plant-herbivore interactions, thereby affecting the efficacy of biological control (Müller-Schärer *et al.* 2004). The Plant Water Stress Hypothesis suggests that increased insect performance during drought may be attributed to increased foliar nitrogen level (Huberty and Denno 2004). Thus, the resource-enemy release hypothesis (R-ERH) predicts that high resource plant species are likely to be susceptible to natural enemies because they have high tissue nutrients that are needed by the insects (Blumenthal 2006). The R-ERH hypothesis combines the enemy release hypothesis (Keane and Crawley 2002) and the resource hypothesis of habitat invasibility (Davis *et al.* 2000). According to these hypotheses, the efficacy of biological control agents on invasive species may be affected by resource availability (Blumenthal *et al.* 2009). This underscores the need to test the efficacy of biological control agents under varying levels of resources.

At times, the lack of success of some biological control programs may be attributable to (i) wrong choice of biological control agents, (ii) releasing biological control agents on wrong

plant species (Myers 2000), (iii) adaptive changes in the target plant in the introduced range (Müller-Schärer *et al.* 2004) or climate incompatibility (asynchronization) that may inhibit establishment of the biological control agents in the new environment (Gassmann and Schroeder 1995; Myers 2000). An example is the initial failure of biological control programs for the leafy and Cypress spurges, *Eurphobia esula* and *Euphorbia cyparissias*, which was a result of morphological variations between spurges from the native and introduced range (Gassmann and Schroeder 1995). Biological control agents that developed on the leafy spurge in the native range were found to be incompatible with the spurges in the introduced range (due to morphological differences), resulting in the failure of the program. Morphological differences between the target plant form and the forms of the same plant in the native range were found to have an impact on the efficacy of agents (Gassmann and Schroeder 1995).

2.5.2 Evolution of Increased Competitive Ability (EICA) hypothesis

Another hypothesis that seeks to explain plant invasiveness is the Evolution of Increased Competitive Ability (EICA) hypothesis (Blossey and Notzold 1995). According to this hypothesis, introduced plants undergo a shift in their resource distribution patterns, maximising allocation to traits that optimise their competitive advantage such as increased growth and fecundity (Callaway and Ridenour 2004; Müller-Schärer *et al.* 2004). Defence is costly to plants (Kant *et al.* 2015; Rhoades 1979), therefore, in the absence of specialist herbivores, invasive plants would naturally allocate more resources to traits that ensure competitiveness (Flory *et al.* 2011; Maron *et al.* 2004). This hypothesis implies that in the absence of specialist phytophagous enemies, selection will favour genotypes with increased competitive abilities, resulting in greater invasiveness potential.

The EICA hypothesis is based on the Growth-Differentiation Balance (GDB) hypothesis which suggests that in nutrient-limiting environments, plants are in a state of "dilemma" whether to allocate resources to growth or defence mechanisms (Herms and Mattson 1992). This trade-off has ecological outcomes that affect the evolution of certain resource allocation patterns in specific environments (Moreira *et al.* 2015). Availability of resources such as water and light also affect invasive plant growth regimes and their biomass allocation patterns (Osunkoya *et al.* 2010a). The EICA hypothesis is closely linked to the ERH (Lau and Schultheis 2015).

As an invasive species occurring outside of its native range, *D. unguis-cati* is potentially excluded from its co-evolved natural phytophagous enemies. Applying the assumptions of the EICA hypothesis, the relative growth rate of *D. unguis-cati* plants from the native range is expected to be significantly lower than the growth rate of the plants from the introduced range (Blossey and Notzold 1995). A study that compared Chinese tallow (*Sapium sebiferum*) plants from the native range (Asia) with those from the introduced range (North America) showed that plants from the introduced range outperformed the ones from the native range (Siemann and Rogers 2003; Zou *et al.* 2008). Several other studies have confirmed the assumptions and predictions of the EICA hypothesis (Blossey and Kamil 1996; Blossey and Notzold 1995; Callaway *et al.* 2008; Gard *et al.* 2013). Because of the limited studies on the growth regime of *D. unguis-cati*, the two co-occurring forms of the weed in Australia should provide a unique system to test the predictions of the EICA hypothesis by comparing their growth patterns and allocation strategies. In the current study, it can be predicted that the more dominant form, SP, will exhibit significantly higher growth related traits than the less common LP form. As both forms are potentially released from natural enemies, significant difference in growth patterns could imply different resource allocation strategies.

2.5.3 The "Ideal Weed" hypothesis (IWH)

Closely knit to the EICA hypothesis is the "Ideal Weed" hypothesis postulated by Baker (1965) and supported in part by Sutherland (2004). This hypothesis correlates certain plant traits with colonization success of invasive species (Rejmánek *et al.* 1995; Rejmanek and Richardson 1996; Smith and Knapp 2001; Sultan and Matesanz 2015). These traits include high reproductive capabilities (Baker 1974; Baskin and Baskin 2001; Frenot and Gloaguen 1994; Hao *et al.* 2009), seed germination (Crawley 1983; Evans and Young 1972), carbon assimilation rates (Baruch and Goldstein 1999; Regnier *et al.* 1988), relative growth rate and phenotypic plasticity (Firn *et al.* 2012; Funk 2008; Richards *et al.* 2006). These functional traits will be discussed in detail below due to their relevance in addressing the question of why SP is more abundant than LP in Australia.

Reproductive traits and colonization success

Reproduction is a fundamental process that ensures establishment of invasive species in their novel range (Hao *et al.* 2009; Pyšek and Richardson 2007). This means that reproduction-

related traits such as breeding systems, flower phenology, self-pollination, efficient seed dispersal strategies, germination rates and propagule size play an important role in plant invasions (Van Kleunen and Johnson 2007; Williamson and Fitter 1996b). Some invasive species provide for reproductive assurance by exhibiting both sexual, asexual and apomictic propagation (Barrett *et al.* 2008 and references therein). Invasive species successfully colonize novel habitats by having high propagule pressure and greater offspring output (Lockwood *et al.* 2005; Mason *et al.* 2008; Simberloff 2009).

Seed germination is one crucial developmental stage in the establishment of species (El-Keblawy and Al-Rawai 2005; Li *et al.* 2008), which governs the ecological success and distribution patterns of plants, including invasive ones (Al Khateeb *et al.* 2010). Time of germination, rate of germination and total germination percentage are measurable characteristics that can enable ecologists to predict the level of success and recruitment of a species in the environment (Ranal and Santana 2006; Soltani *et al.* 2002). High versatility in reproductive characteristics can be selected for because the evolutionary success of any organism is directly proportional to the number of individuals in existence, the extent of their reproductive output and the range of habitats they can survive and proliferate in (Baker 1965; Baker 1974).

Seeds must be exposed to the appropriate environmental cues (moisture, light, temperature and substrate pH. conditions) to initiate the process of germination (Frankland and Taylorson 1983; Idikut 2013; Li *et al.* 2008; Mandák 2003; Nandula *et al.* 2006; Probert *et al.* 1985; Rokaya and Münzbergová 2012; Serrano-Bernardo *et al.* 2007). Conditions required for germination usually vary between species and at times interact to either promote or inhibit germination (Li *et al.* 2008). Invaders exhibit a higher level of germination plasticity in response to environmental conditions (Baker 1974; Culliney 2005; Sultan 2000; Tinoco-Ojanguren *et al.* 2016; Wainwright and Cleland 2013), thus increasing the range of niches they can exploit (Flint and Palmblad 1978; Li *et al.* 2015; Wen 2015). Flexible or plastic development is a vital character of a weed's "general purpose genotype" (Baker 1965). Temperature is one of the most significant factors that affect both the germinability and germination rates of seeds (Idikut 2013; Mijani *et al.* 2013). Some plants germinate within a narrow temperature range, while others have wider temperature amplitude for germination. Availability of light and its intensity, especially during periods of soil water availability, also

play a significant role as a trigger for germination in most plant species (Al Khateeb *et al.* 2010; Frankland and Taylorson 1983; Li *et al.* 2015; Milberg 1997; Wang *et al.* 2016).

Light is needed for seedlings to photosynthesise and so the interactive effects of temperature and light substantially contribute to promoting germination during conditions that enhance the survival of the seedling stage (Baskin and Baskin 2001; Milberg 1997; Milberg *et al.* 1996; Thompson and Grime 1983). Life history strategies, including seed germination cues, may be shaped by natural selection. Alternative strategies between species or varieties along life history trait gradients could be adaptive to maximize fitness under different environmental conditions (Easton and Kleindorfer 2008).

Polyembryony is the formation of extranumerary embryos in single seeds (Trapero *et al.* 2014; Webber 1940), and has been shown to increase propagule pressure of some invasive species in novel environments (Blanchard *et al.* 2010). Such embryos arise from either apomictic (asexual) or amphimictic (sexual) processes (Mendes-Rodrigues *et al.* 2012). Occurrence of polyembryony is ascertained through emergence of multiple seedlings from a single seed during germination (Firetti-Leggieri *et al.* 2013). Although little is known about the ecological consequences of polyembryony (Blanchard *et al.* 2010), any process that increases the number of individuals to the next generation is advantageous as it adds to the propagule pressure (Catford *et al.* 2009). However, some evidence suggests that polyembryony may be disadvantageous due to competition between polyembryonic siblings from early developmental stages through to seedling establishment (eg Mendes-Rodrigues *et al.* 2012). Although polyembryony is widely reported in angiosperms, it is prevalent in only a few families, including Myrtaceae, Cactaceae, Rutaceae, Anacardiaceae and Bignoniaceae (Ganeshaiah *et al.* 1991). In the family Bignoniaceae, polyembryony has been reported in species like *Handroanthus ochraceus*, *H. chrysotrichus* (Bittencourt Jr and Moraes 2010), *Anemopaegma acutifolium*, *A. arvense*, *A. glaucum* and *A. scabriusculum* (Firetti-Leggieri *et al.* 2013).

Functional traits and invasiveness

Multiple factors are responsible for invasion success (Blumenthal 2005; Daehler 2003; Lamarque *et al.* 2011; Leffler *et al.* 2014; Leung *et al.* 2004; MacDougall *et al.* 2009). It is generally agreed that the correlation of certain plant performance traits to invasiveness is context-dependent (Moravcová *et al.* 2015). However, a pattern of relatedness to invasiveness has been reported for some plant traits (Pyšek and Richardson 2007). Mostly, fitness traits such

as leaf area ratio, carbon assimilation, growth rate and shoot/root allocation show marked differences between evidently invasive and non-invasive species (Grotkopp *et al.* 2002; Moravcová *et al.* 2015; van Kleunen *et al.* 2010b).

Invasive species were shown to have higher values of traits like SLA (Burns 2006; Lake and Leishman 2004), RGR (Dawson *et al.* 2011), and more biomass allocated to organs like stems resulting in taller plants (Gallagher *et al.* 2015; Stanisci *et al.* 2010; van Kleunen *et al.* 2015). High specific leaf area (SLA) is an important plant trait that facilitates capture of photosynthetically active radiation (PAR) and is often associated with high RGR (Grotkopp and Rejmánek 2007), although other studies have not found that trend (see, for example, Garcia-Serrano *et al.* 2005; Osunkoya *et al.* 2010a). RGR is also influenced by net assimilation rate (NAR) which is the rate of dry weight increase per unit of leaf area (Grotkopp *et al.* 2002; Vernon and Allison 1963). Although SLA is often related to leaf area and leaf dry mass (Wilson *et al.* 1999), it also closely interacts with the internal anatomy of the leaf (Osunkoya *et al.* 2014; Sefton *et al.* 2002), thus directly affecting regulation of water (H_2O) and carbon dioxide (CO_2) profiles (Evans 1999) within plants. Therefore RGR is an important trait that incorporates aspects of plant morphology, anatomy and physiology that can be quantified and compared between invaders and non-invaders (Osunkoya *et al.* 2014).

It is generally agreed that fast growing plants are more likely to be invasive than slow growing ones (Blumenthal and Hufbauer 2007; Lake and Leishman 2004; Richardson 1998). Higher values of growth related traits in invasive species compared to less invasive ones imply different strategies for capture and efficient use of resources such as light, carbon, nitrogen and moisture (Gallagher *et al.* 2015). Because resources are almost always limiting in the environment (Cordell *et al.* 1998), efficient use of limiting resources by invasive species can enhance their colonizing success (Pattison *et al.* 1998a). In disturbed environments, species that are better able to exploit fluctuating resources will likely invade the system (Cordell *et al.* 1998; Leffler *et al.* 2014; van Kleunen *et al.* 2010b). The apparent differences in performance and fitness related traits (e.g. SLA, RGR and reproductive output) between invasive vs. non-invasive species suggest that trait studies are important in understanding invasions. This is in contrast to suggestions that traits are not useful in our understanding of invasion success (Davis *et al.* 2011; Richardson and Ricciardi 2013; Thompson and Davis 2011; Thompson *et al.* 1995; Valéry *et al.* 2013; van Kleunen *et al.* 2011).

Most studies aimed at understanding differences in traits associated with invasion success have used native species as control plants (Muth and Pigliucci 2006; Osunkoya *et al.* 2010a; Osunkoya *et al.* 2014). The limitation of this approach is that these native species may potentially or already be invasive elsewhere (Burns 2004; Drenovsky *et al.* 2008; van Kleunen *et al.* 2010b) or may even have invasive capacity in their native domain (e.g. Valery *et al.* 2009). Some native species used in a comparative study by Godoy *et al.* (2011) and Leishman *et al.* (2010) were reported to be invasive in other parts of the world. Muth and Pigliucci (2006) argue that some native species were shown to have invasive tendencies in their native range. These findings imply that introduced versus native species comparisons may not always be informative (but see Blossey and Notzold 1995; Callaway and Ridenour 2004; Dawson *et al.* 2015b; Keane and Crawley 2002; van Kleunen *et al.* 2011). Other studies have also shown that traits do not always differ between invasive and non-invasive species (Meiners 2007; Smith and Knapp 2001; Thompson *et al.* 1995). An assessment of 122 species including non-native invasive and native species that occupy disturbed areas did not find significant differences in most traits previously associated with successful colonizers (Leishman *et al.* 2010). There could also be a bias in choosing highly competitive invasive species and comparing them with known weak native competitors in pairwise experiments (Vila and Weiner 2004) or comparing phylogenetically non-related species (Burns 2006).

Thus, our understanding of invasiveness traits could be enhanced by comparing related non-native species of varying levels of colonization success (Kolar and Lodge 2001; Muth and Pigliucci 2006; van Kleunen *et al.* 2010b). This approach is a direct test of determinants of successful colonization and has previously yielded insightful results (e.g Blackburn and Jeschke 2009; Küster *et al.* 2008; van Kleunen *et al.* 2010a).

How do phenotypic plasticity and integration influence colonization success?

It is commonly agreed that phenotypic plasticity is a potentially vital mechanism that drives colonization success, thus enhancing plants invasiveness (Chun *et al.* 2007; Nicotra *et al.* 2010; Sultan and Matesanz 2015). Phenotypic plasticity is the ability of an individual organism or a genotype to express varying traits/phenotypes in response to a range of environmental conditions (Bradshaw 1965; Pigliucci 2001; Richards *et al.* 2006). Single genotypes may adjust their biochemistry, physiology and morphology in response to biotic or abiotic cues (Agrawal 2001; Schlichting 1986; Via and Lande 1985). Ideally, phenotypic plasticity should be determined in genetically identical replicates exposed to a continuum of

environments. However, because phenotypic plasticity is taken in a general sense, mean plasticity indices across similar genotypes is permissible (Callaway *et al.* 2003; Valladares 2003).

Plastic response of plants to fluctuating environmental conditions can increase the average fitness of a species across environments. Plasticity of traits in response to environmental changes must be adaptive in order to increase the average fitness of species (Lande 2015; Osunkoya *et al.* 2010a; Schlichting 1986). Adaptive plasticity could drive differences in ecological and geographical distribution of closely related taxa, including invasive and non-invasive species (Nicotra *et al.* 2010). Widespread species are expected to exhibit high phenotypic plasticity when compared to plants with restricted distribution (e.g., non-invasive but naturalised species) (Firn *et al.* 2012; Funk 2008; Ghalambor *et al.* 2007). High trait plasticity is associated with colonization success as it encourages rapid spread into environmentally heterogeneous habitats (Godoy *et al.* 2011). This is made possible by enhancement of the ecological niche breadth of potential colonizers (Richards *et al.* 2006), which has been found to enhance plant invasions (Blumenthal and Hufbauer 2007; van Kleunen and Fischer 2008). Association of the niche breadth and range size suggest that plants that can utilize a wide range of resources will have greater success in spread (Brown 1984). Exploitation of micro-niches ensures that plastic species maintain fitness and competitiveness under changing conditions (Corliss and Sultan 2016).

Analyses (meta- and experimental) of invasive vs non-invasive pairs have shown that in general, invasive species express greater phenotypic plasticity than their non-invasive counterparts. For example, a meta-analysis of 75 pairs of invasive/non-invasive species found that invasive species were nearly always more plastic than their counterparts, although this plasticity did not always translate to fitness (Davidson *et al.* 2011). Individual studies comparing either phylogenetically related invasive/non-invasive plants have also shown that invasive species exhibit greater phenotypic plasticity (see Chun *et al.* 2007; Claridge and Franklin 2002a; Claridge and Franklin 2002b; Flory *et al.* 2011; Geng *et al.* 2006; Lamarque *et al.* 2013; Molina-Montenegro *et al.* 2012; Pan *et al.* 2006; Sultan and Matesanz 2015; Wainwright and Cleland 2013).

In contrast, several other studies did not find any significant differences in the plasticity of important traits between invasive and non-invasive species (Douhovnikoff *et al.* 2016). In an experiment involving 105 plant species, Dostál *et al.* (2016) found that the less invasive

species were even more plastic than invasive ones (also see Ruprecht *et al.* 2013). In another experiment using 1152 seedlings from 8 native and 8 invasive populations of the Manitoba maple species to compare plastic responses to nutrient availability, Lamarque *et al.* (2013) did not find any increased plasticity from the invasive population. They concluded that their results could be an indication that invasive species have pre-adapted plasticity, as opposed to the widely held view of post-introduction evolution of phenotypic plasticity (see Lande 2015; Schlichting 1986). This view is similar to that of Firn *et al.* (2011) who observed that species that have invasive tendencies in their native range are likely to be invasive in their introduced range. Another meta-analysis by Palacio-López and Gianoli (2011) concluded that invasive species do not display greater phenotypic plasticity than non-invasive species.

Further research into the role of plasticity in determining invasion success has concluded that it is trait values, and not trait plasticity that determine superior performance by invasive species (Matzek 2012). For example, a comparison of 20 pairs of invasive/native species by Godoy *et al.* (2011) found similar high levels of trait plasticity between invasive and native species. So they concluded that rather than considering trait plasticity in isolation as a determinant of colonization success, trait means should always be considered side by side. It is vital to mention that the experiment by Godoy *et al.* (2011) had some limitations in that some 'native' species used were known to be 'invasive' elsewhere (also see Muth and Pigliucci 2006; van Kleunen *et al.* 2010a). Nevertheless, Godoy *et al.* (2012) demonstrated that indeed trait means were more important than trait plasticity in determining performance of invasive species. An earlier review of the importance and limits of phenotypic plasticity by Valladares *et al.* (2007) indicate that sometimes plastic responses could be maladaptive and therefore reduce general plant fitness.

In light of the conflicting conclusions of both meta-analyses and experimental investigations on phenotypic plasticity and its association with invasiveness, Lande (2015) attempts to proffer an explanation. The author argues that conflicting conclusions result from the complexity of the adaptive processes, which may involve an initial rapid increase in plasticity of a species upon introduction, followed by a slow genetic assimilation ending in reduced plasticity later in the invasion continuum. So the different possible outcomes of these processes, the heterogeneity among plasticity experiments and varying residence times of invasive species account for inconsistent outcomes of plasticity studies (Bock *et al.* 2015; Lande 2015).

Other studies argue that phenotypic integration of functional traits enhance colonization of environmentally heterogenous habitats. Phenotypic integration is an estimation of the number of trait pairs that are significantly correlated in response to environmental conditions (Luo *et al.* 2015; Osunkoya *et al.* 2014). The higher the number of correlated trait pairs, the more integrated the species is, which enables it to efficiently cope with changing environments (Pigliucci 2003; van Kleunen and Fischer 2005). In theory, a more integrated phenotype results in adaptive response to the environment, which would constrain mal-adaptive phenotypic plasticity (Godoy *et al.* 2012; Waitt and Levin 1993). Mal-adaptive phenotypic plasticity is costly to plants because it decreases their fitness (Valladares *et al.* 2007; van Kleunen and Fischer 2005).

2.6 Biology and ecology of two forms of *D. unguis-cati*

The two forms of *D. unguis-cati* with different levels of abundance seem appropriately placed to be used as case study using a trait based framework (Downey and Turnbull 2007; Shortus and Dhileepan 2011). Previous studies have found that the two forms showed differences in some life history traits. In a field experiment using plants generated from tubers, Taylor and Dhileepan (2012) found that the LP form produced greater total dry mass (hence higher RGR) than the SP form. Osunkoya *et al.* (2009) also noted some differences in stem density of genets and ramets between the two forms in field samples, but decried lack of data on growth rates and reproductive capacity for the two forms.

The germination traits of *D. unguis-cati* have not been adequately investigated. A seed bank ecology study by Vivian-Smith and Panetta (2004a) suggests that this species does not have a persistent seed bank; it also shows low seed longevity, usually less than 12 and 1% at 1 year for soil-surface (< 1 cm depth) and 5 cm depth buried seeds, respectively. The same study also indicates that *D. unguis-cati* seed germination requirements are non-specific, germinating over a wide range of light and temperature conditions. However, taking into consideration the current knowledge of existence of two forms of this species (Shortus and Dhileepan 2011), this study (i.e Vivian-Smith and Panetta 2004b) was limited because it only refers to the SP form. Therefore the current study seeks to compare germination traits of both LP and SP under varying temperature and light regimes (Buru *et al.* 2014).

To date, the biological control program for *D. unguis-cati* has involved the introduction of a leaf-sucking tingid, *Carvalhotingis visenda* (Hemiptera: Tingidae) (Dhileepan *et al.* 2007b), a leaf tying moth, *Hypocosmia pyrochroma* (Lep., Pyralidae, Chrysauginae) (Dhileepan *et al.* 2007a) and a leaf mining jewel beetle, *Hylaeogena jureceki* (Coleoptera: Buprestidae) (Dhileepan *et al.* 2013). Both *C. visenda* and *H. pyrochroma* are relatively well established in Queensland (Dhileepan 2012; Dhileepan *et al.* 2010). However, the occurrence of two forms of *D. unguis-cati* provides opportunity to test intraspecific diversity in target weeds on the preference and performance of introduced biological control agents. Some evidence suggests that marked variations in plants may affect plant-herbivore interactions (Biere and Verhoeven 2008; Caswell and Reed 1976; Müller-Schärer *et al.* 2004). In Australia, all the three biological control agents have been used for both forms of *D. unguis-cati*. However, no comparative studies have been conducted to determine the efficacy of introduced biological agents on both forms of *D. unguis-cati.*

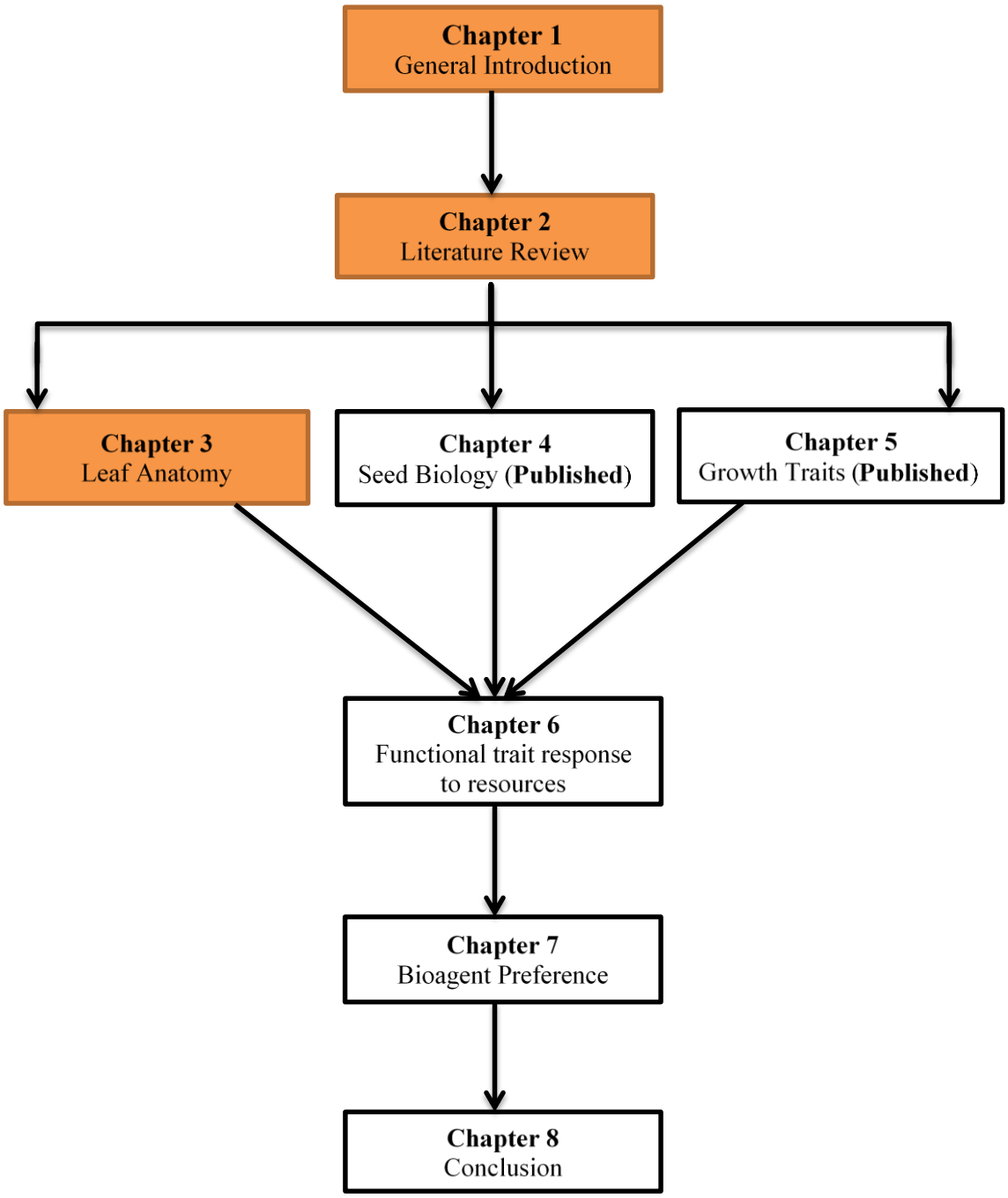
A population biology assessment of *D. unguis-cati* variability in both the native and introduced ranges showed high genetic variation in the native range with over 27 haplotypes. In the introduced range, only 4 haplotypes were identified and about 98% of the specimen from the introduced range matched a single haplotype from Paraguay (Prentis *et al.* 2009). According to Dhileepan (2012) this dominant haplotype in the introduced range is likely to be the SP (also see Boyne *et al.* 2013a). Although the same study by Prentis *et al.* (2009) identified a second haplotype in Australia, they did not differentiate the two forms of *D. unguis-cati*, so it is not clear whether the second haplotype corresponds to the LP or not. Dhileepan (2012) also observes that from herbarium records and field surveys, LP is widely distributed in the native range, occurring from Mexico, Nicaragua, Costa Rica, Columbia to Brazil. Therefore, it is expected that locally adapted *D. unguis-cati* natural enemies from the source of the dominant haplotype, in this case Paraguay, will make the most effective biological control agents (Gassmann and Schroeder 1995; Goolsby *et al.* 2006; Keane and Crawley 2002; Prentis *et al.* 2009).

It is noteworthy that, all the biological control agents for *D. unguis-cati* being tested in Australia were initially collected on the LP form, from sites in Brazil and Argentina in 2002 (Dhileepan *et al.* 2007a; Dhileepan *et al.* 2013; Dhileepan *et al.* 2007b; King *et al.* 2011b; Snow *et al.* 2006; Treviño *et al.* 2006). So could it be that the biological control agents that are

used to control SP in Australia are insects of LP in the native range? Moreover, host-specificity tests for control agents did not take into account the existence of two forms of *D. unguis-cati* in Australia (Dhileepan *et al.* 2007a; Dhileepan *et al.* 2013; Dhileepan *et al.* 2007b). SP was the only form of *D. unguis-cati* majorly used for testing the suitability of the agents for release (pers.com Dhileepan). This gap in the knowledge suggests the need to investigate whether the biological control agents are equally effective in managing both forms of *D. unguis-cati in* Australia.

2.7 Conclusion

The literature review presented here has identified knowledge gaps about *D. unguis-cati* that this study will address. Although there have been some studies on *D. unguis-cati*, most of the studies did not factor in the two morphologically distinct forms that exhibit different distribution patterns. Therefore, it is not often clear which form is being referred to whenever conclusions are drawn from such studies (e.g. Prentis *et al.* 2009; Vivian-Smith and Panetta 2004b). Studies that have compared *D. unguis-cati* with other non-invasive congeners may also be misleading, considering that there might be variation between the forms. This present study will quantify a series of seed biology, leaf and whole plant traits associated with resource acquisition and performance between the two forms of *D. unguis-cati*. We predict that SP, the more prevalent form will have higher performance traits, greater plasticity, produce more biomass and exhibit superior carbon assimilation rates than LP. If our hypothesis is accepted, we will argue that this pattern of development could confer greater competitiveness on SP. We also predict that the more abundant form in the native range, LP, will be the preferred one by the biological control agents. If this hypothesis is accepted, we will also argue that different control measures should be considered for the two forms in Australia. The large number of traits measured in this study will be analysed by multivariate techniques (e.g. MANOVA and ordinations) to summarise how similar or different the two forms of *D. unguis-cati* are. The outcome of this study will also determine whether we argue for a separation of the two forms into different functional species or two extremes of the same species (see Boyne *et al.* 2013a).


Chapter 1
General Introduction
Chapter 2
Literature Review
Chapter 3
Leaf Anatomy
Chapter 4
Seed Biology (Published)
Chapter 5
Growth Traits (Published)
Chapter 6
Functional trait response
to resources
Chapter 7
Bioagent Preference
Chapter 8
Conclusion

Chapter 3: Leaf anatomy and micro-morphology of the two forms of *Dolichandra unguis-cati*

3.1 Abstract

The aim of this study was to compare leaf anatomical characters between long pod (LP) and short pod (SP) forms of *Dolichandra unguis-cati*. Leaves of the two forms were obtained from localities around the Greater Brisbane area and fixed in formalin-acetic-acid-alcohol (FAA) solution. Leaf anatomy was studied using paraffin embedding, microtomy and staining with toluidine blue (TBO) whilst surface micromorphology was studied using scanning electron microscopy and leaf replicas. The leaves of both forms were amphistomatic and SP had significantly higher stomatal density than LP. Simple unicellular and multicellular hairs occur on both surfaces of LP while only the unicellular type was found in SP. Two types of glandular nectaries (patelliform and peltate) were observed in both forms. The smaller peltate nectaries were more common than the larger patelliform type in both forms, and SP had a higher frequency than LP for both nectary types. The palisade layer of SP was found to be thicker than that of LP. Differences in indumenta (hairiness) and nectary frequency could have implications for biological control of the two forms. Differences in anatomical traits such as types of epidermal hairs and calcium oxalate crystals have taxonomic implications for LP and SP. Differences in stomatal density and palisade mesophyll thickness could have ecophysiological implications for the two forms. The present finding provides valuable information that will inform further research on the two forms of *D. unguis-cati* in Australia.

3.2 Introduction

This study is the first attempt to compare anatomical and micro-morphological leaf characters of the two forms of *D. unguis-cati*. The aims of this study are to document and compare leaf anatomy and micro-morphology between LP and SP from field collected materials. We believe that our results will act as baseline for future morpho-anatomical and

ecophysiological studies that will compare these two forms, and could help explain ecophysiological differences between the two forms (See Chapters 5 and 6).

A study by Osunkoya *et al.* (2014) found significant differences in hair density, thicknesses of the different leaf layers and palisade/spongy mesophyll ratios between SP and a native vine, *Pandorea jasminoides*. The study found significant differences in anatomical trait plasticity and coordination between the invasive group and native vines, with significant correlations between stomatal complexes and plant physiology (Osunkoya *et al.* 2014). Plants often exhibit differences of functional traits that affect capture and utilisation of resources and adaptability to varying environmental conditions (Sultan 2000; Zanne and Falster 2010). For example, the Hawaiian tree, *Metrosideros polymorpha*, is known to occur across a wide range of moisture variable habitats because of its ability to adjust the thickness of the water-holding leaf hypodermis (Cordell *et al.* 1998). The leaf presents an appropriate model for investigating linkages between anatomy and physiology because it is the main site through which the plant interacts with the external gaseous environment to facilitate photosynthesis (Mediavilla *et al.* 2001; Sankar *et al.* 2016).

Some anatomical and micro-morphological characters such as stomatal position, composition of the vascular system of the midrib, number of palisade and spongy mesophyll layers are of taxonomic value in Bignoniaceae (Firetti-Leggieri *et al.* 2013; Gama *et al.* 2013; Ogundipe and Wujek 2004), and therefore may shed more light into the existing taxonomic confusion between LP and SP (see Boyne *et al.* 2013a). Foliar nectaries (also known as extra-floral nectaries) are ubiquitous in Bignoniaceae (Gama *et al.* 2013; Ogundipe and Wujek 2004). Approximately 90% of genera belonging to the family Bignoniaceae bear secretory foliar nectaries, more than 60% of which have similar morphology and anatomy (Elias and Gelband 1976). The type and distribution of foliar nectaries could be taxonomically informative but may also have implications for biological control of the two forms. Foliar nectaries are known to produce sweet exudates that attract ants and therefore play a significant role in the insect-plant mutualistic relationships (Galetto and Bernardello 1992; Koptur 1979). Gentry (1974) observed that most species in the family Bignoniaceae attract ants, which in turn act as repellents to other herbivores. This has a potential to jeopardize biological control efforts, especially if ants deter natural herbivores used as biological control agents (do Nascimento and Del-Claro 2010).

LP and SP has been shown to differ in leaf morphology (Boyne *et al.* 2013a; Shortus and Dhileepan 2011). The variation in morphology of different forms of a species may have negative consequences for biological control in the introduced range (Gassmann and Schroeder 1995). This is because biological control agents may only be able to recognise, survive, establish and exert maximum damage on certain cultivars or varieties (Cilliers and Neser 1991; Zalucki *et al.* 2007). Moreover, differences in leaf morphology and anatomy may have ecophysiological performance implications for plants. It is not known whether there are differences in leaf anatomy and micromorphology between LP and SP.

3.3 Materials and Methods

Sampling strategy

Fresh leaf samples were collected from populations of the LP and SP in the Greater Brisbane area, Queensland, Australia. Leaves of both forms were obtained from the following sites: South Bank (27°55'S, 153°01'E), Ipswich Forest Reserve (27°32'S, 152°42'E), Bardon (27°30' S, 152°41'E), Carindale (27°30'S, 152°41'E) and Sherwood (27°30'S, 152°59'E). Mature and young leaves from randomly selected branches were collected for this investigation. Fresh samples were fixed in formalin-acetic-acid-alcohol solution (FAA) for at least 24 hours, and stored in 70% ethanol (Boyne *et al.* 2013b; Johansen 1940). A single leaflet per plant was selected and four plants were randomly studied from each site.

Leaf anatomy and micromorphology

Methodology for fixation, processing, embedding, sectioning and staining was the same as that of Boyne *et al.* (2013b). Leaf clearings were prepared by immersing a small piece (approximately 2 cm^2) in 10% KOH for 48 hours. Samples were then immersed in 7% Sodium hypochlorite (NaClO) for a minimum of 2 hours until they turned transparent (Retamales *et al.* 2014). Cleared leaflets were washed in distilled water, stained with Safranin O and mounted with lactoglycerol (Retamales and Scharaschkin 2015).

Leaf replicas were used to study the frequency and distribution of foliar nectaries, pavement cells and stomatal complexes (Hilu and Randall 1984; Nepi *et al.* 1996). Leaf

impressions or epidermal replicas were prepared by smearing a thin but uniform layer of nail varnish on both the adaxial and abaxial leaf surfaces (Chen *et al.* 2001). The nail varnish was left to dry for at least 30 minutes and then gently pulled off using a transparent sticky tape. The tape with the leaf impression was mounted on a glass slide for observation. Slides were observed using a Nikon SMZ800 light microscope (Nikon Eclipse 50i compound, Nikon Corporation, Tokyo, Japan), while images were captured using Nikon NIS-Elements Imaging software. The NIS Element Imaging Software was used to measure epidermal, palisade and spongy mesophyll thickness from images. Stomatal density was calculated by dividing the number of stomatal pores by the area examined, and an average calculated for the whole leaflet. The number of foliar nectaries and stomatal complexes per unit area was used to determine distribution of each type of nectary and stomata from both the adaxial and abaxial leaf surfaces.

Surface micro-morphological traits were studied using scanning electron microscopy (SEM). FAA-fixed leaflet samples were dehydrated through a series of ethanol concentrations and dried using hexamethyldisilazane (HMDS) (Pathan *et al.* 2010). Subsequently, samples were treated in the same way as described in Retamales *et al.* (2014). Voucher specimens of plant samples collected from each site have been lodged with the Queensland Herbarium (BRI), voucher number AQ522127-AQ522128. Voucher specimens obtained from Oxley and Carindale localities correspond to those lodged by Boyne *et al.* (2013a).

Terminology and analyses

Basic anatomical terminology followed Esau (1953). The terminology used to describe anatomical characters such as stomatal traits, trichomes, midrib, midvein and mesophyll cells followed that of Paliwal (1970) and Firetti-Leggieri *et al.* (2013). Foliar nectaries were identified and described following Elias and Gelband (1976), Ogundipe and Wujek (2004) and Gama *et al.* (2013). Analysis of variance (ANOVA) was used to compare means of epidermal thickness, mesophyll layer thicknesses, nectaries, epidermal hairs and stomatal density using SPSS package (IBM SPSS Statistics for Windows, version 22.0). As randomly selected leaflets were examined in this study, and no differences were found between the leaflets, subsequently they will be referred to as leaves in this chapter.

3.4 Results

The leaf anatomy and micromorphology of LP and SP will be presented tissue-by-tissue from the dermal, ground to vascular tissue systems.

3.4.1 Dermal tissue system

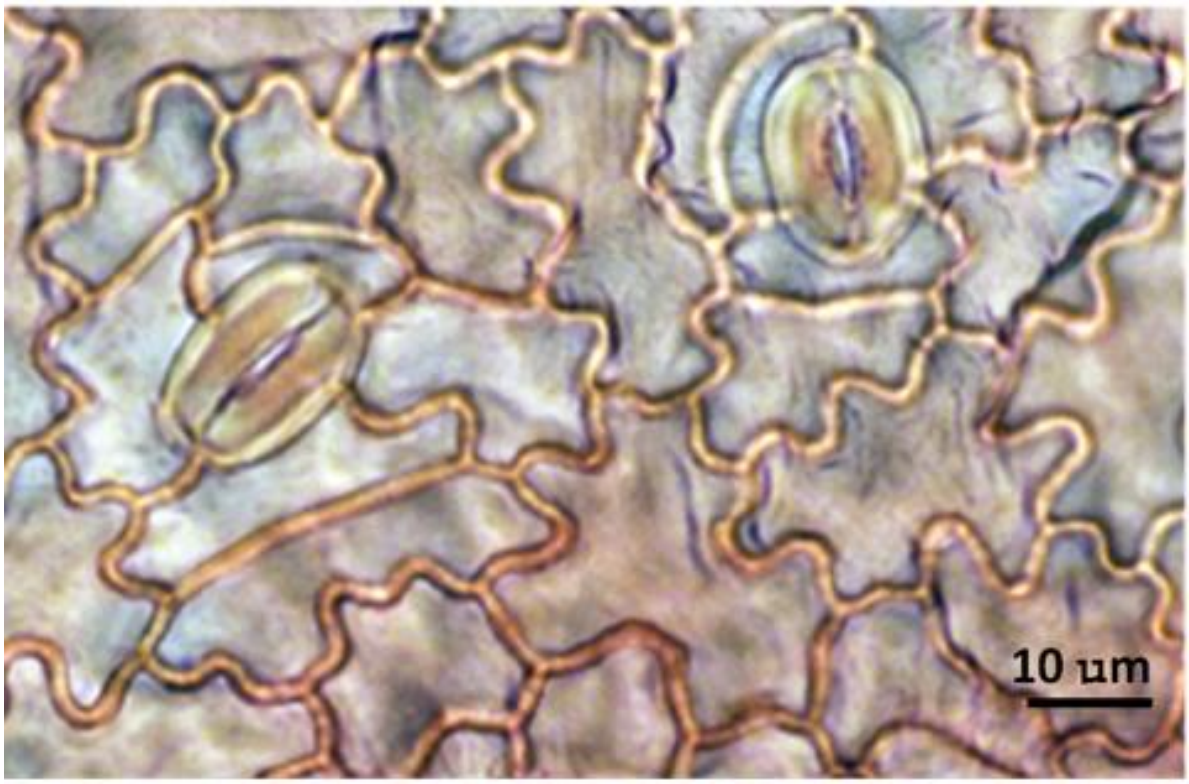


Figure 3.1. Light micrograph of cleared paradermal leaf sections of *D. unguis-cati* (SP) showing sinuous pavement cells and anisocytic stomatal complexes. Both LP and SP have similar pavement cells and stomatal complexes.

Epidermal cells

The epidermis is a single (uniseriate) layer on both the adaxial and abaxial surfaces in LP and SP except at the midrib. Epidermal pavement cells of both forms are irregular or sinuous in shape **(Figure 3.1)**. The adaxial and abaxial epidermal layers of SP are significantly thicker than those of LP **(Figure 3.2a)**. Although statistically insignificant, the adaxial epidermis is slightly thicker than the abaxial epidermis in LP, whilst both the adaxial and abaxial epidermal layers are similar in thickness in SP **(Table 3.1)**. The cuticle is ornamented on both surfaces of

both forms, with a more striking ornamentation on the adaxial surface of the LP but with no such difference observed in SP.

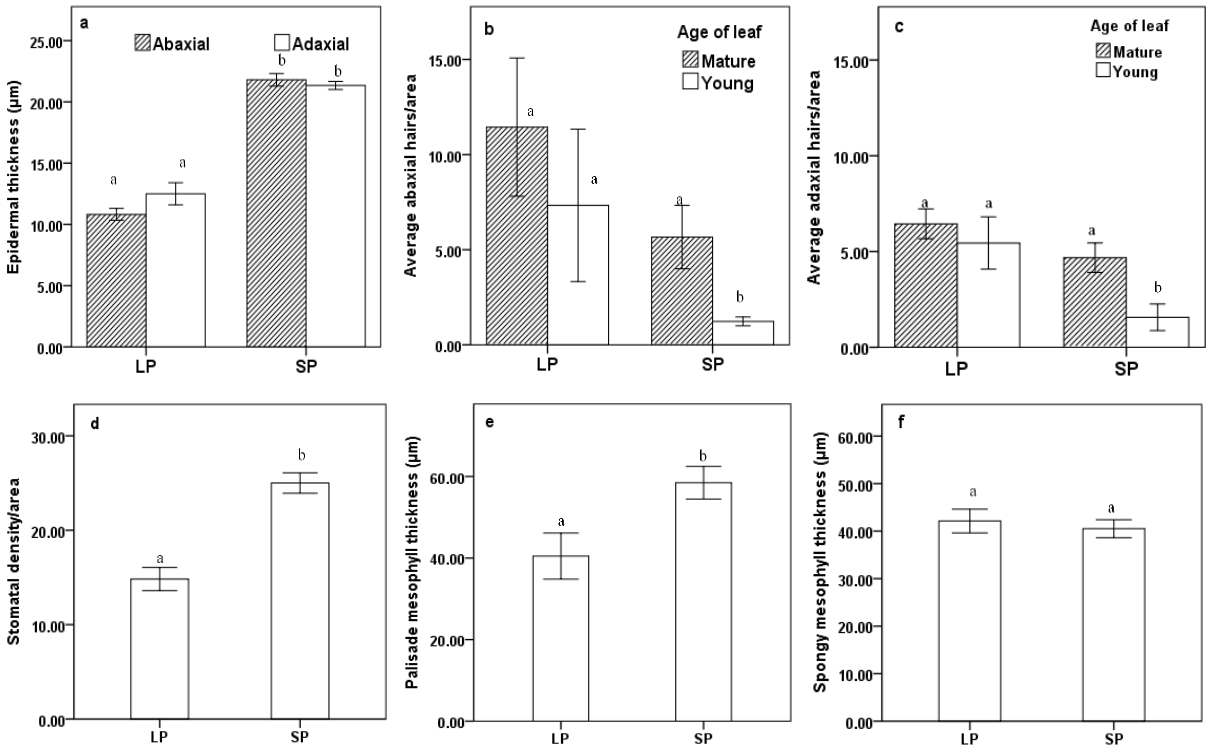


Figure 3.2. Comparison of mean anatomical traits (± SE) of both forms (LP and SP) of *D. unguis-cati*. (a) Abaxial and adaxial epidermal thickness (µm); (b) Abaxial hair densities in mature and young leaves; (c) Adaxial hair densities in mature and young leaves; (d) Stomatal density; (e) Palisade mesophyll thickness (µm) and (f) Spongy mesophyll thickness (µm). Similar letters above the bars indicate insignificant differences between forms

Stomatal complexes

Both LP and SP have amphistomatic leaves with higher stomatal density on the abaxial surface. Stomata are randomly located on the surface of the leaves with no particular pattern of occurrence. The stomatal density in SP is significantly higher than that of LP ($F_{1, 3} = 27.593$, $P<0.001$; **Figure 3.2d**). Both forms have anisocytic stomata **(Figure 3.1)**. Stomata appear to have sub-stomatal chambers that are separated by layers of spongy parenchyma cells. The stomata are sparsely distributed on the adaxial surface in both forms of *D. unguis-cati*.

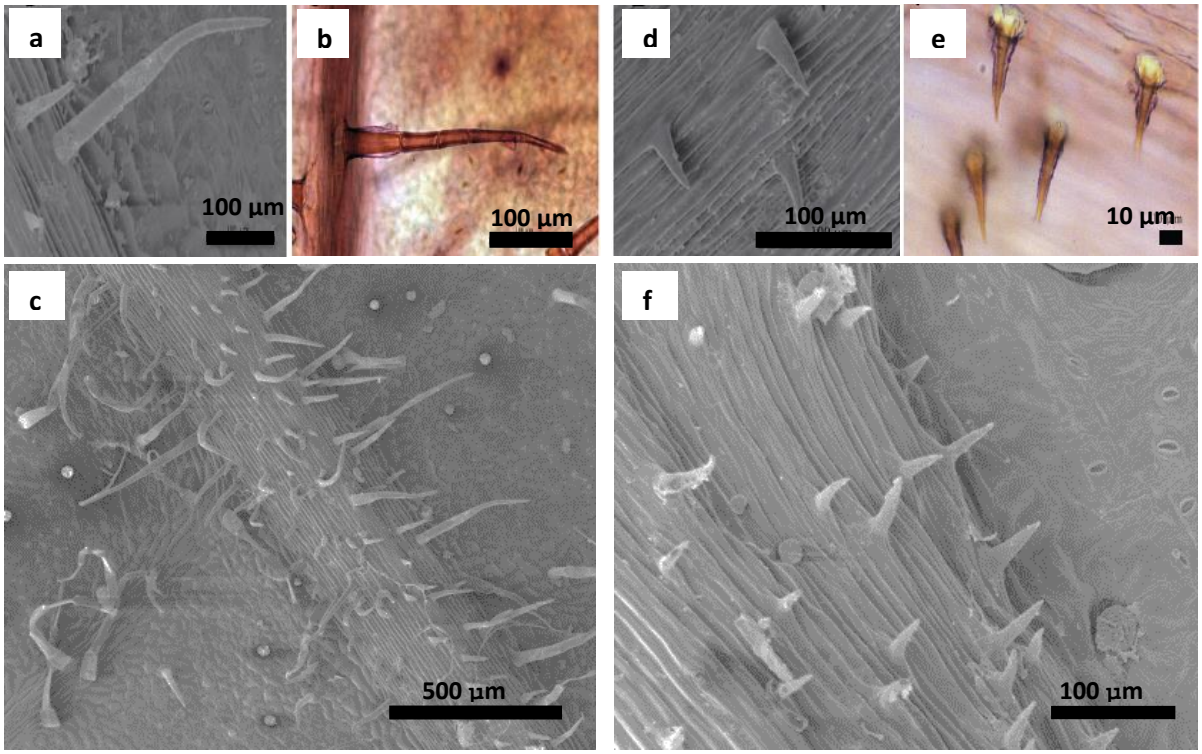


Figure 3.3. Scanning electron microscopy (SEM) and light micrographs of unicellular and multicellular hairs in the two forms (LP and SP) of *D. unguis-cati*. a-c: LP; d-f: SP; a-b: multicellular hairs in LP; c: distribution of both unicellular and multicellular hairs; d-e: unicellular hairs; f : distribution of unicellular hairs along a major vein.

Epidermal hairs

Two different types of hairs (also called non-glandular trichomes) were observed in *D. unguis-cati*. Both hair types have an acute apex (**Figure 3.3)**. Unicellular and uniseriate hairs, originate from single epidermal pavement cell **(Figure 3.3c, d, f)** whilst multicellular and uniseriate hairs, originate from two or more epidermal pavement cells **(Figure 3.3a, b, e)**. Both types of hairs are found in LP **(Figure 3.3a, c)** while only the unicellular type is found in SP, occurring sparsely along the primary and secondary veins (**Figure 3.3f)**. The distributions of the hairs on the adaxial surface differ significantly between the two forms ($F_{1, 3}$ = 8.984, P<0.02). LP has numerous and occasionally very dense multicellular hairs distributed over the entire lamina on both surfaces (**Figure 3.3c)**. The unicellular types of hairs appear to only occur along the veins in LP.

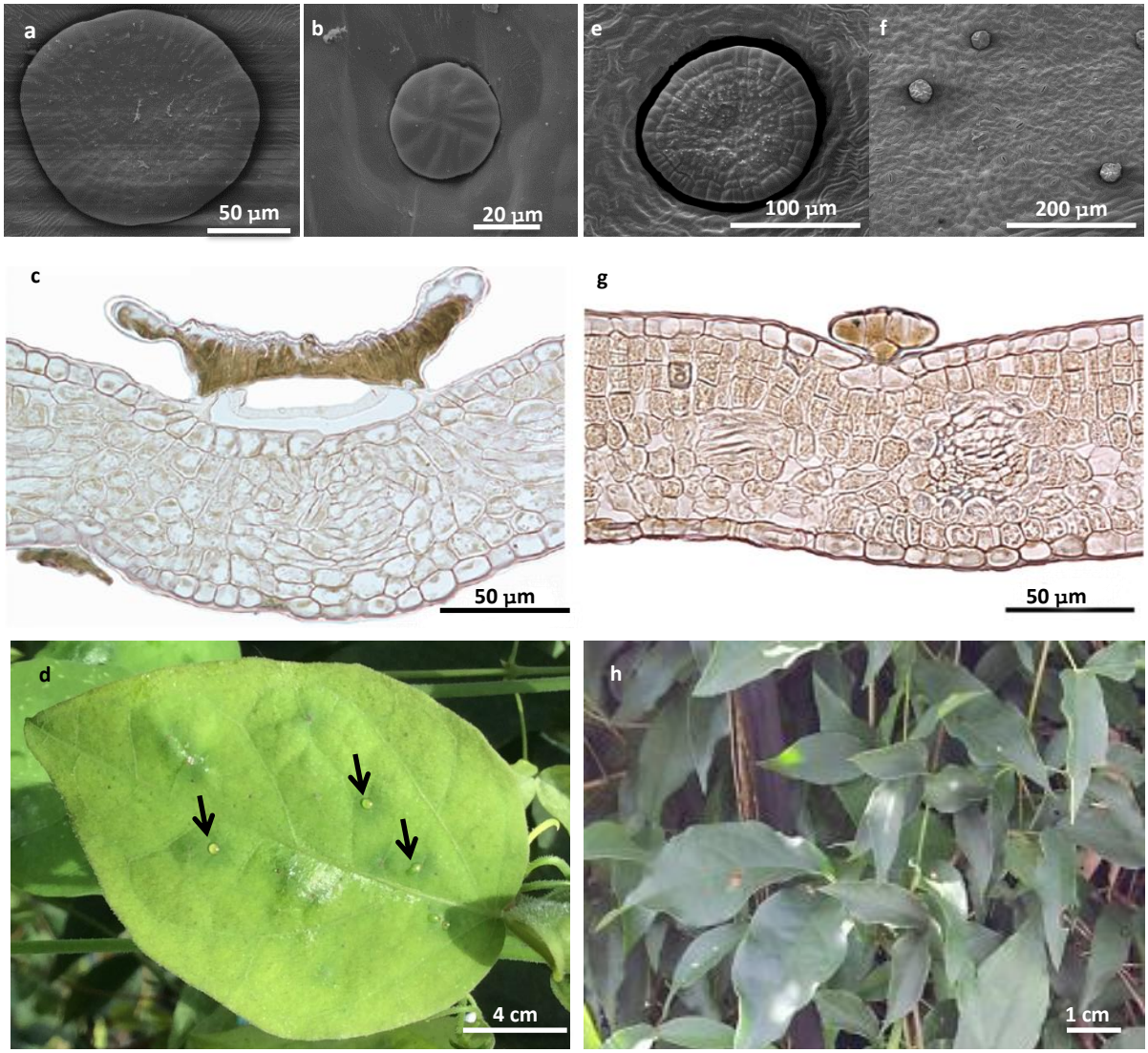


Figure 3.4. Scanning electron micrographs (a-b, e-f), transversal light micrographs (c, g) and pictures (d, h) of foliar nectaries in two forms (LP and SP) of *D. unguis-cati.* a-d: LP; e-h: SP; a: patelliform nectary in LP; b: peltate nectary in LP; c: transversal view of a patelliform nectary; d: LP leaflet showing prominent exudates (indicated by arrows); e: patelliform nectary in SP; f: peltate nectary in SP; g: transversal view of a peltate nectary; h: SP leaflets without obvious nectaries or exudates.

Foliar nectaries

Two types of foliar nectaries (also glandular trichomes or extra-floral nectaries) occur in *D. unguis-cati*. The larger patelliform nectaries are discoidal in appearance and usually 130 ± 10 µm in diameter. They are comprised of ~50 cells arranged in a single layer on a single large foot cell (**Figure 3.4a, c, e)**. The smaller peltate nectaries are 25 ± 5 µm in diameter and comprised of 8-10 isodiametric cells arranged in a single layer with a single small foot cell **(Figure 3.4b, f, g)**.

There is a significant difference in the distribution of nectaries (both types) on the adaxial and abaxial surfaces and between LP and SP, indicated by a significant interaction of form and leaf surface ($F_{1, 7}$ = 19.970, P<0.001). Both types of nectaries occur more frequently in SP than LP, but for each form, there are more nectaries on the abaxial rather than the adaxial surfaces **(Table 3.1)**. Significant interactions of form and nectary type ($F_{1, 3}$ = 42.981, P<0.001) indicate that the distribution of the two types of nectaries differs significantly between LP and SP, regardless of the leaf surface. Peltate nectaries occur more frequently than patelliform nectaries in both forms ($F_{1, 7}$ = 160, P<0.001). LP nectaries appear to exude prominent nectars that attract insects **(Figure 3.4d),** but such prominent nectars were not observed in SP **(Figure 3.4h)**.

3.4.2 Ground tissue system

Palisade mesophyll

Lamina of LP is predominantly dorsiventral but certain parts of the leaflets present isolateral symmetry (bifacial). Where the leaflets present isolateral symmetry, the abaxial palisade is always thinner that the adaxial one. LP consists of 3-4 layers of the adaxial palisade mesophyll cells **(Figure 3.5b)**. The SP leaflets are only dorsiventral, consisting of 4-7 layers of the palisade mesophyll cells **(Figure 3.5e)**. Overall, the palisade mesophyll of the SP is significantly thicker than that of the LP ($F_{1, 3}$ = 8.042, P<0.003; **Table 3.1; Figure 3.2e)**. Age of the leaf did not have a significant effect on the palisade mesophyll thickness ($F_{1, 3}$ = 3.926, P<0.090) and did not interact with form either ($F_{1, 3}$ = 0.029, P<0.90).

Spongy mesophyll

LP presents a spongy mesophyll consisting of compactly packed cells with small intercellular spaces in between the cells. The spongy mesophyll cells of LP generally appear round in shape, mostly 1-3 layers except in regions where there appears to be abaxial palisade mesophyll (isolateral symmetry), in which case there is only one or two layers of the spongy mesophyll cells **(Figure 3.5b)**. The spongy mesophyll layer in SP is less compact with large intercellular spaces. The cells are smaller, irregularly shaped and are generally three layers consistently **(Figure 3.5e)**. There is no significant difference in spongy mesophyll thickness between LP and SP ($F_{1, 3}$ = 0.213, P<0.700; **Figure 3.2f)**.

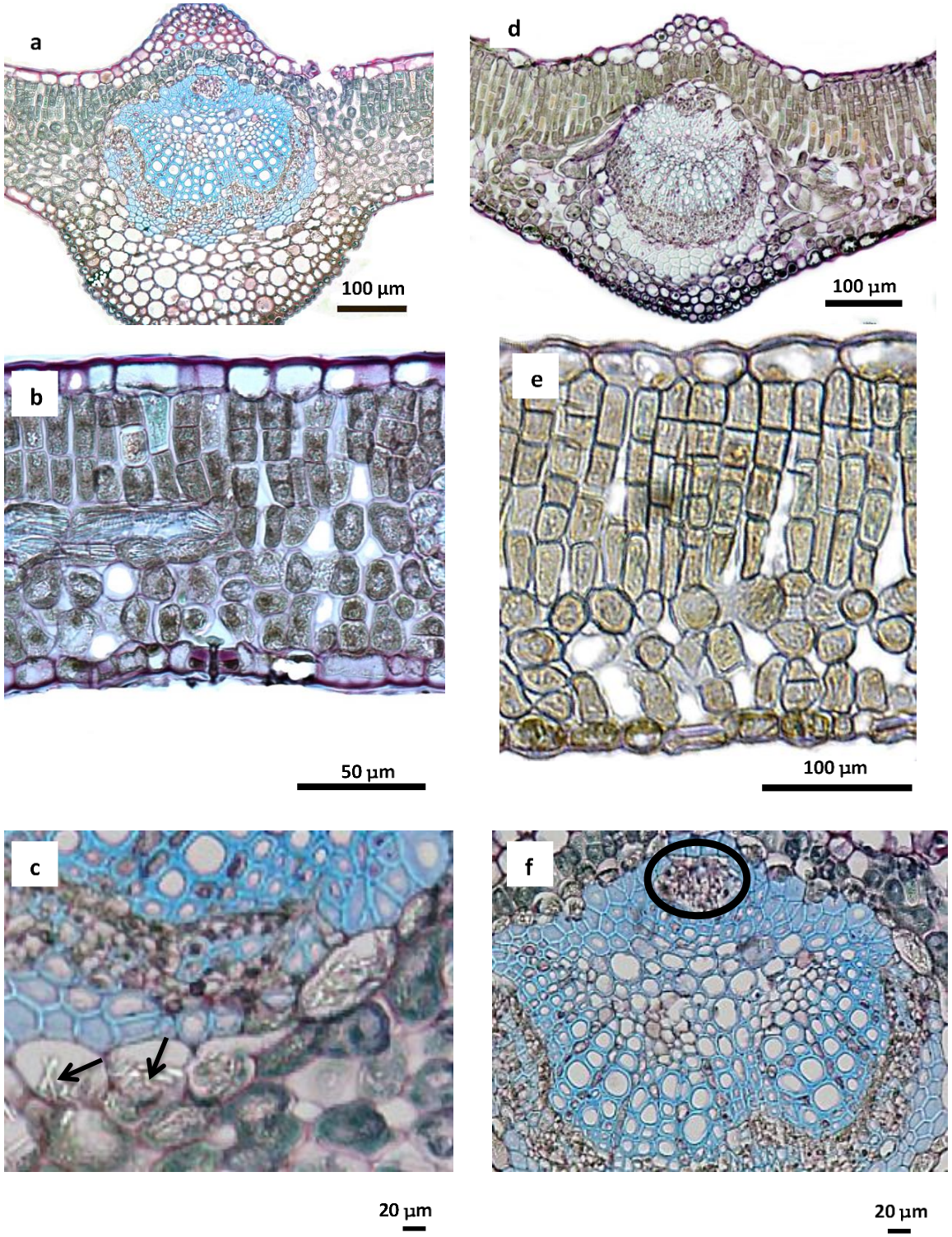


Figure 3.5. Transverse light micrographs showing leaf transverse sections of the two forms (LP and SP) of *D. unguis-cati*. a-c,f: LP; d-e: SP; a: midrib section in LP; b: mesophyll region in LP; c: calcium oxalate crystals in the bundle sheath cells of LP shown by arrows; d: midrib section in LP; e: mesophyll region in SP; f: the location of adaxial phloem in both LP and SP indicated by the black polygon.

3.4.3 Vascular tissue system

Midrib

In LP, the midrib is abaxially and adaxially dislocated and thus producing a highly prominent rib **(Figure 3.5a)**. The midrib has multiseriate layers of epidermal cells on both surfaces around the vascular system. Two to four layers of large epidermal cells appear on the adaxial surface while there are only two layers of smaller epidermal cells on the abaxial surface. There are 5-7 layers of sub-epidermal collenchyma cells on the abaxial side of the midrib. The palisade mesophyll layer continues above the midrib, at which stage it is composed of only two layers **(Figure 3.5a)**. Calcium oxalate crystals were observed in the bundle sheath cells surrounding the midrib in LP but were not observed in SP **(Figure 3.5c)**.

The midrib is much less prominent in SP **(Figure 3.5d)**. The midrib has a uniseriate layer of epidermal cells occur on the adaxial surface above the midrib and a double layer of smaller epidermal cells on the abaxial surface below the vascular system. There are 2-3 layers of hypodermal collenchyma cells on the abaxial side of the midrib. Two to four layers of the palisade parenchyma cells continue above the midrib. The palisade layer that occurs above the midrib is thicker in SP than in LP **(Figure 3.5a, d)**. Both LP and SP generally have a U-shaped collateral vascular bundle with phloem strands surrounded by sclerenchyma fibres. Both forms have an additional adaxial phloem **(Figure 3.5a, d, f)**.

Table 3.1 Mean (± SE) anatomical characters of LP and SP. Summary ANOVA refers to F- and P-values of a one-way analysis of variance. F subscripts represent degrees of freedom. P values that are in bold show significant difference between LP and SP for the trait

Anatomical trait	Form		Summary ANOVA
	LP	SP	*F*-value, *P*-value
Epidermal thickness (μm)			
Abaxial	10.822 ± 0.481	21.802 ± 0.512	$F_{1, 3}$ = 244.4, ***P*<0.001**
Adaxial	12.502 ± 0.900	21.342 ± 0.330	$F_{1, 3}$ = 85.054, ***P*<0.001**
Mesophyll thickness (μm)			
Palisade layer	40.487 ± 5.642	58.453 ± 4.019	$F_{1, 3}$ = 8.042, ***P*<0.05**
Spongy layer	42.122 ± 2.497	40.518 ± 1.892	$F_{1, 3}$ = 0.213, *P*>0.01
Stomatal density (mm^2)	14.833 ± 1.222	25.003 ± 1.080	$F_{1, 3}$ = 27.593**, *P*<0.001**
Hair density (mm^2)			
Abaxial	9.389 ± 2.587	3.450 ± 1.245	$F_{1, 3}$ = 8.984, ***P*<0.01**
Adaxial	5.944 ± 0.737	3.122 ± 0.836	$F_{1, 3}$ = 4.400, *P*>0.05
Foliar nectary density (mm^2)			
Patelliform (abaxial)	0.250 ± 0.001	0.750 ± 0.002	$F_{1, 3}$ = 13.404, ***P*<0.001**
Patelliform (adaxial)	0.139 ± 0.002	0.167 ± 0.001	$F_{1, 3}$ = 0.104, *P*>0.05
Peltate (abaxial)	13.500 ± 0.734	19.250 ± 0.995	$F_{1, 3}$ = 34.897**, *P*<0.001**
Peltate (adaxial)	4.306 ± 0.233	5.889 ± 0.125	$F_{1, 3}$ = 24.077, ***P*<0.001**

3.5 Discussion

This study demonstrates that leaf anatomical and micro-morphological features are a valuable source of information that could differentiate two forms of *D. unguis-cati*. Anatomical characters measured in this study have been previously shown to have taxonomic (Firetti-Leggieri *et al.* 2013; Ogundipe and Wujek 2004), physiological (Araque *et al.* 2009) and weed management applications (do Nascimento and Del-Claro 2010). The stomatal density, epidermal cells, the ratio of palisade and spongy mesophyll and arrangement of the midrib of the two forms of *D. unguis-cati* were found to be significantly different in this study. The leaf anatomy and arrangement of the different types of cells is important because all the gaseous exchange and photochemistry that drive ecophysiology occur here, especially in the mesophyll (Bernacchi *et al.* 2002; Mediavilla *et al.* 2001). Although important, anatomical structures are still poorly understood and the linkage with ecophysiological performance is rarely made (Osunkoya *et al.* 2014).

Given that leaves often adapt to the environment by changing leaf lifespan, specific leaf area (SLA) and nutrient content, it should be expected that leaves will have anatomical

correlation to performance (Berry and Downton 1982; Somavilla *et al.* 2014). SLA is a measure of biomass investment per leaf unit area and thus a function of leaf thickness (Feng *et al.* 2008; Wilson *et al.* 1999). From the differences in epidermal and palisade mesophyll thickness, it is clear that SP leaves are significantly thicker than for LP. This study has found particularly interesting that the palisade mesophyll of SP consisting of more layers (4-7) than LP (3-4). Considering that SP has significantly narrower leaves than LP (Shortus and Dhileepan 2011), investing more on the photosynthetic apparatus by SP ensures its physiological performance. Chapter 6 of this thesis reports that LP and SP have similar SLA, although LP has significantly higher leaf area than SP. As differences in SLA can indicate variable resource capture strategies (Westoby *et al.* 2002), the LP and SP forms may have different light capture strategies. Osunkoya *et al.* (2014) demonstrated that there is a positive correlation between some anatomical characters and ecophysiological performance, and the correlations differed significantly between invasive and non-invasive species. Thicker leaves are often associated with species that are adapted to high light conditions (Palliotti and Cartechini 2015; Terashima *et al.* 2001).

This study found SP to exhibit significantly higher stomatal density than LP, which could affect physiological performance differently in the two forms. Stomata are the gateway for gaseous exchange between the plant and atmosphere and they control transpiration rate (Hetherington and Woodward 2003; Meidner and Mansfield 1968). An increased stomatal density was found to correlate positively with water use efficiency (WUE) (Zhao *et al.* 2015) and physiological indices such as carbon assimilation, transpiration and stomatal conductance (Xu and Zhou 2008). Because stomatal density is highly plastic (Fraser *et al.* 2009; Zhao *et al.* 2015), more studies are required to compare the two forms under different resource levels, especially light.

Although the same types of foliar nectaries occur on both forms of *D. unguis-cati*, the variation in distribution between the two forms is informative. SP has a higher frequency of foliar nectaries on both surfaces than LP. SP particularly shows a dense occurrence of small peltate nectaries on the abaxial surface of the leaves. Foliar nectaries have been found to secrete sugary exudates to attract ants that protect foliage against herbivory (Elias and Gelband 1976; Koptur 1979). Clustering of small peltate nectaries all over the SP leaf is advantageous as it increases the surface area that may be covered by the ants, thereby protecting the whole leaf. The exudates released by the nectaries may establish an important mutualistic insect-plant

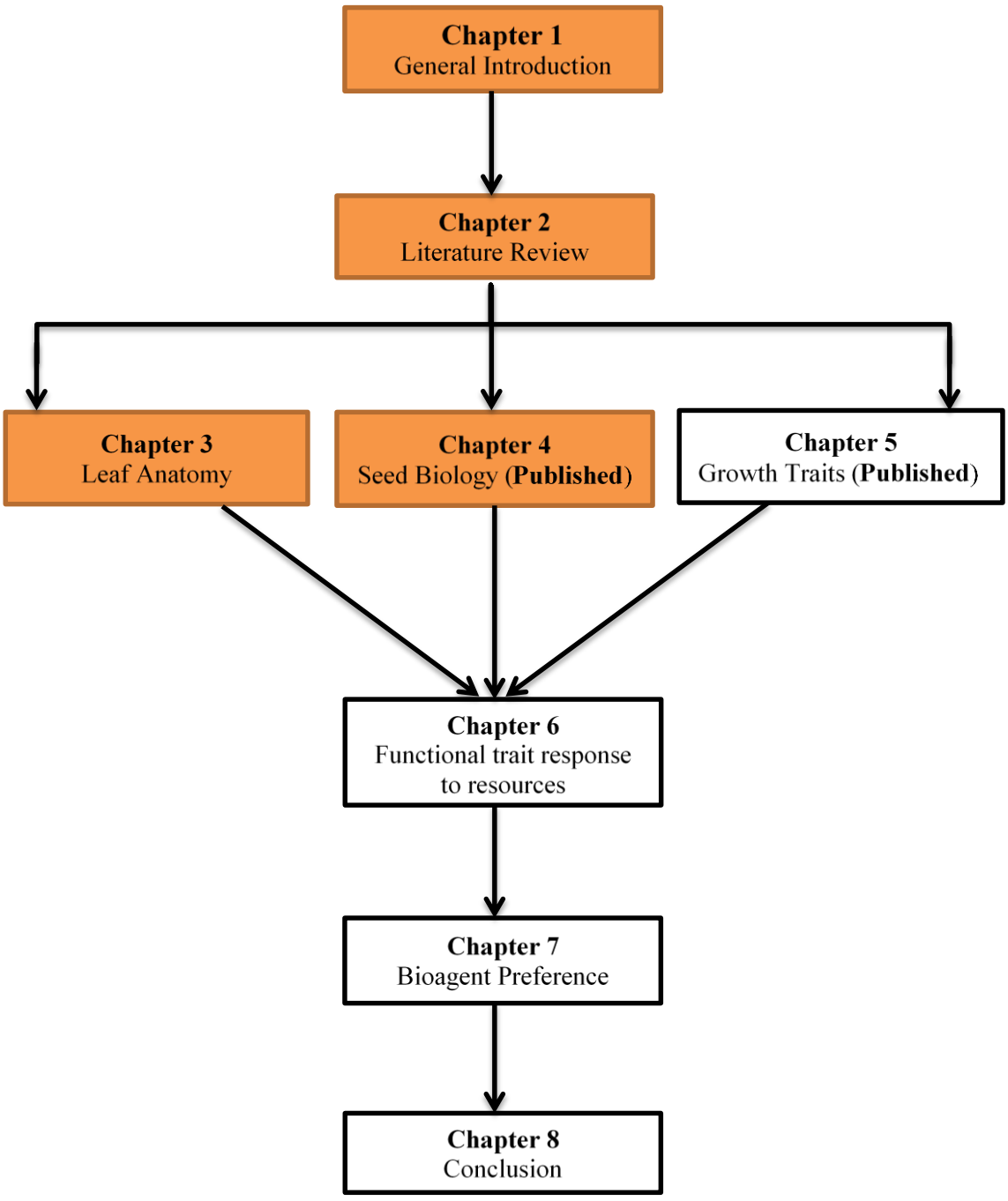
relationship. Facultative mutualism is common in colonising and invasive plants with foliar nectaries (Koptur 1979). So an invasive species may be successful in the novel environment by attracting biotic defense mechanisms such as ants in non-native locations. For a weed species, this may have negative effects on biological control of variable forms that have differing frequencies of nectaries. Koptur (1979) observed that nectaries of the weedy vetches, *Vicia sativa* were visited by the ant, *Iridomyrmex humilis* and plants from which nectaries had been removed suffered greater damage from herbivores than control plants. This is an indication that the ants that are attracted by exudates from nectaries protect host plant foliage from attack by herbivores.

As the same types of foliar nectaries occur in both forms of *D. unguis-cati*, this trait cannot be used to differentiate the two forms taxonomically. The observed differences in the foliar nectary frequency between the two forms could be a plastic response attributable to effects of micro-habitats prevailing where leaflets where obtained (e.g Mondor *et al.* 2006). Although SP showed higher frequency of nectaries, during this study, prominent exudates and ants were only observed on the LP. It could be that the two forms produce different nectar types (but see Koptur 1979). More investigations on the function of nectaries in LP and SP are required to find out if there is any benefit of exudates and ant visitation in *D. unguis-cati.*

The findings of this study also significantly corroborate the suggestions by Boyne *et al.* (2013a) that these two forms may either be two species or two extremes of the same species. Variation of certain characters such as foliar nectaries, midrib, midvein, proportion of different mesophyll cells and histo-chemicals has been used previously to delimit species in Bignoniaceae (Elias and Gelband 1976; Firetti-Leggieri *et al.* 2013; Gama *et al.* 2013; Ogundipe and Wujek 2004). Presence of calcium oxalate crystals in the mesophyll cells of the LP and their absence from the cells of the SP is taxonomically significant in Bignoniaceae (see Firetti-Leggieri *et al.* 2013). Although species of the family Bignoniaceae are known to exhibit intraspecific variation in leaf morphology, it is not known whether this variation extends to the anatomical level (Gentry 1976).

Conclusions

This is the first study that describes the anatomy and micromorphology of the two forms of *D. unguis-cati* in Australia. The study provides a baseline for future comparative plasticity studies between the two forms under different resource conditions. Significant differences in various anatomical characters such as foliar nectary frequencies found in this study also have implications for biological control strategies. The study provides valuable information that may be useful for the taxonomic resolution of the two forms. Based on our findings and previous findings by Boyne *et al.* (2013a), we hypothesise that LP and SP are not the same species and recommend a phylogenetic study to resolve their status.



Statement of Contribution: The first author was responsible for conducting the experiments, data collection, data analysis and interpretation, and writing the manuscript. The co-authors were involved in refining the methodology, providing statistical support and editing of the manuscript. Co-authors also provided logistical support for conducting the experiments.

Chapter 4: Germination biology and occurrence of polyembryony in two forms of cat's claw creeper vine, *Dolichandra unguis-cati* (Bignoniaceae): implications for its invasiveness and management.

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4.1 Abstract

Cat's claw creeper, *Dolichandra unguis-cati* (L.) Lohmann (syn. *Macfadyena unguis-cati* (L.) Gentry) is a major environmental weed in Australia. Two forms ('long' and 'short' pod) of the weed occur in Australia. This investigation aimed to evaluate and compare germination behaviour and occurrence of polyembryony in the two forms of the weed. Seeds were germinated in growth chambers set to 10/20 °C, 15/25 °C, 20/30 °C, 30/45 °C and 25 °C. Germination and polyembryony were monitored over a period of 12 weeks. For all the treatments in this study, seeds from the short pod form exhibited significantly higher germination rates and higher occurrence of polyembryony than those from the long pod form. Seeds from the long pod form did not germinate at the lowest temperature of 10/20 °C; in contrast, those of the short pod form germinated under this condition, albeit at a lower rate. Results from this study could explain why the short pod form of *D. unguis-cati* is the more widely distributed form in Australia, while the long pod form is confined to a few localities.

The results have implication in predicting future ranges of both forms of the invasive *D. unguis-cati*, as well as inform management decisions for control of the weed.

Keywords Macfadyena unguis-cati, plant sexual reproduction, plant invasion, propagule pressure, seed ecology, woody vine.

4.2 Introduction

Plant invasions result in environmental degradation (Pyšek and Richardson 2010), heavy financial costs (Pimentel *et al.* 2005) and loss of biodiversity (Wilson 1989). Understanding plant traits contributing to invasiveness may thus help in determining the best way to manage invasive species (Burns 2004). Many biotic and abiotic hypotheses have been proposed to explain why some species become invasive (Baker 1974; Blossey and Notzold 1995; Keane and Crawley 2002). Species-specific traits such as high specific leaf area, competitiveness, greater morphological and physiological plasticity than co-occurring non-invasive species, niche pre-emption, and release from natural enemies in the novel environment determine plant invasiveness (Callaway and Ridenour 2004; Osunkoya *et al.* 2010b). There is an increasing evidence that propagule pressure (size, number of individuals introduced, temporal and spatial patterns of arrival and establishment in a novel ecosystem) also play a major role in driving invasion success (Catford *et al.* 2009; Lockwood *et al.* 2005; Simberloff 2009).

Reproductive strategies of invasive plants also play a significant role at all the stages of the invasion process. Versatility in reproductive strategies ensures variable range of environments into which the invasive plants can spread and proliferate (Baker 1974). Time-to-germination initiation and rate of germination are measurable characteristics that can be used to predict the success of any species in a given environment (Soltani *et al.* 2002). Most plant species germinate optimally within a narrow range of environmental conditions, but the ability to germinate under different environmental conditions (*i.e.,* germination plasticity) can be an adaptation to maximize fitness, especially for invasive species in novel environments (Baker 1974; Lockwood *et al.* 2005). An important cue for seed germination is the ambient temperature, especially during periods of soil water availability (Mijani *et al.* 2013). The interactive effects of temperature and light conditions may also substantially influence

germination and thus enhance the survival and establishment of the seedling stage (Baskin and Baskin 2001).

Some plant species exhibit a rare phenomenon of polyembryony i.e., the formation of extranumerary embryos in single seeds (Trapero *et al.* 2014; Webber 1940). Polyembryony has been shown to further increase the propagule pressure of a species in novel environments (Blanchard *et al.* 2010). Such embryos arise from either apomictic (asexual) or amphimictic (sexual) processes (Mendes-Rodrigues *et al.* 2012). The occurrence of polyembryony is ascertained through emergence of multiple seedlings from a single seed during germination (Firetti-Leggieri *et al.* 2013). Although little is known about the ecological consequences of polyembryony (Blanchard *et al.* 2010), any process that increases the number of individuals to the next generation is advantageous as it adds to the propagule pressure (Catford *et al.* 2009). However, some evidence suggests that polyembryony may be disadvantageous due to competition between polyembryonic siblings from early developmental stages through to seedling establishment (eg Mendes-Rodrigues *et al.* 2012). Although polyembryony is widely reported in angiosperms, it is prevalent in only a few families, including Myrtaceae, Cactaceae, Rutaceae, Anacardiaceae and Bignoniaceae (Ganeshaiah *et al.* 1991). In the family Bignoniaceae, polyembryony has been reported in *Handroanthus ochraceus*, *H. chrysotrichus* (Bittencourt Jr and Moraes 2010), *Anemopaegma acutifolium*, *A. arvense*, *A. glaucum* and *A. scabriusculum* (Firetti-Leggieri *et al.* 2013).

Cat's claw creeper, *Dolichandra unguis-cati* (L.) Lohmann (syn. *Macfadyena unguis-cati (*L.) Gentry) (Bignoniaceae) is a native of the Greater and Lesser Antilles, Mexico, South and Central America to Argentina, including Trinidad and Tobago (Gentry 1976). It was introduced to Australia as an ornamental plant in the late 1800s, but has since naturalised and is considered a major environmental weed (Downey and Turnbull 2007). *D. unguis-cati* has recently been listed as a Weed of National Significance (WoNS) in Australia (Dhileepan *et al.* 2013). It is regarded as an environmental weed in other parts of the world, such as southern and central Africa, Asia, North America and parts of Europe (Dhileepan 2012) and is included in the Global Invasive Species Database (GISD) (De Poorter and Browne 2005).

D. unguis-cati is a woody vine (liana) of riparian areas, where it smothers the tree canopies and can cause trees to collapse due to its immense biomass (Batianoff and Butler 2003). It also creates thick mats on forest floors that smother low vegetation and hamper seedling recruitment (Downey and Turnbull 2007). This growth pattern transforms natural

habitats into monospecific stands, resulting in loss of floral biodiversity and changes in soil biota and chemistry (Osunkoya *et al.* 2011; Perrett *et al.* 2012). *D. unguis-cati* regenerates sexually, through the production of numerous papery seeds, and asexually (vegetatively) by production of subterranean tubers (Downey and Turnbull 2007; Osunkoya *et al.* 2009).

Two morphologically and phenologically distinct forms of *D. unguis-cati* occur in Australia (Shortus and Dhileepan 2011). These forms have been informally referred to as long pod (LP) and short pod (SP) based on their average fruit length at maturity (LP 70 cm; SP 30 cm). LP and SP have, on average, 120 and 61 seeds per fruit at maturity, respectively. The fruits are capsules but have been informally referred to as pods. Seeds of both forms are two-winged, papery and flattened/oblong in shape, 10 - 18 mm long, 4 - 6 mm wide. The average seed biomass is not significantly different between the two forms (Shortus and Dhileepan 2011). SP is the more prevalent form in Australia and occurs in eastern Queensland and northeast New South Wales, while LP is only known from a few isolated localities in southeast Queensland (Boyne *et al.* 2013a). SP is the form of *D. unguis-cati* that is regarded as an environmental weed in different parts of the world (Boyne *et al.* 2013a; Prentis *et al.* 2009). LP does not appear to be as invasive as SP as it occurs in only a few localities in southeast Queensland. However, the cause for this difference in the level of prevalence between the two forms is not known, and one potential cause could be differences in their seed biology. Seed germination dynamics of both forms of *D. unguis-cati* have not been adequately studied. The only study on the seed bank ecology of SP (Vivian-Smith and Panetta 2004b) found it to have low seed longevity, usually less than 12% at 1 year for soil-surface (< 1 cm depth) and 1% for buried seeds (5 cm depth). The same study also inferred the occurrence of polyembryony to be approximately 40% due to emergence of multiple seedlings from single seeds (Vivian-Smith and Panetta 2004b) but did not confirm the presence of polyembryony using established methods (e.g., radicle emergence and seedling separation) nor differentiate between different classes of polyembryony (e.g., twins, triplets). It is not known whether polyembryony occurs in LP and at what frequency. Interestingly, a study from the native range, concluded that *D. unguis-cati* did not exhibit any polyembryony (Firetti-Leggieri *et al.* 2013).

The aims of this study were to determine whether there are differences in seed germination behaviour of the two forms of *D. unguis-cati* by 1) documenting the range of temperature and photo-regime over which seeds of will germinate; 2) confirming if

polyembryony occurs in both forms; and 3) determining the frequency and classes of polyembryony in the two forms.

4.3 Materials and Methods

Acquisition and Storage of Seeds

Seeds of the long pod (LP) and the short pod (SP) forms of *D. unguis-cati* were collected during the fruiting months of 2013 from various sites around the greater Brisbane area in southeast Queensland, Australia. SP seeds were obtained from the following infestation sites: South Bank (27°55'S, 153°01'E), Ipswich Forest Reserve (27°32'S, 152°42'E), Chelmer (27°47'S, 152°58'E), Bardon (27°30'S, 152°41'E) and Boonah (27°60'S, 152°41'E). LP seeds were collected from Carindale (27°30'S, 152°41'E), Bardon (27°30'S, 152°41'E) and Sherwood (27°30'S, 152°59'E). Fewer sites were sampled for LP because this form is less prevalent than SP, and flowering (and consequently fruit formation) does not occur every year (Shortus and Dhileepan 2011). Seeds were collected from fruits at the time of dehiscence to ensure maturity of the seeds (Downey and Turnbull 2007). Once collected, seeds were stored for two weeks at room temperature in paper envelopes placed in containers with silica gel to ensure they remained dry before the commencement of germination assays. For the purposes of this experiment, seeds from different sites were pooled together to ensure adequate sample sizes, although we recognise that there could be differences in germination behaviour between individual plants and amongst sites for a given form.

Experimental Design

Seed germination: Seeds were physically screened to ensure they were firm and intact. Those that appeared not to have viable embryonic content and/or were damaged by insects were not included in the germination assays. Seeds were sterilised by soaking in 1% sodium hypochlorite (NaOCl) for 5 minutes, then rinsed in water for 3 minutes (Mijani *et al.* 2013). Sterilised seeds were placed in 15 cm diameter petri dishes lined with 2-3 layers of 15 cm Whatman filter paper (No. 1) moistened with distilled water. They were subsequently exposed to varying temperature regimes in growth chambers (model ADAPTIS A1000; Conviron Ltd.,

USA) at the Queensland University of Technology (QUT) in Brisbane, Australia. Germination conditions were set to (i) cool (10/20 °C), (ii) moderate (15/25 °C), (iii) warm (20/30 °C) and (iv) hot (30/45 °C) for 12 hours at each alternate temperature. Seed germination took place in light/dark conditions (12-hour photo-period) or constant 24-hours dark. When a 12-hour photo-period was applied, the higher temperature corresponded with the presence of light. Most of the temperature regimes followed the conditions applied by Vivian-Smith and Panetta (2004b) and also reflect the night-day temperature fluctuations in the distribution range of *D. unguis-cati* in Australia. Two additional treatments included constant room temperature (25°C) with 12-hour light/dark and 24-hour dark. Fifteen replicates of 20 seeds each were used for each form in each treatment. Germination data were recorded every seven days for 12 weeks, after which the assay was terminated because no more appreciable germination was observed. After the 12^{th} week, the ungerminated seeds were physically checked and found to be mostly rotten with no visible viable embryo, except for those in the low temperature (10/20 °C). A dim light was used to examine the seed germination in the continuous darkness treatment. Each seed was considered to have germinated with the emergence of one or more radicles (Baskin and Baskin 2001). Total germination percentage was calculated from the total number of germinated seeds divided by the total number of seeds. Germination rate index (GRI) was calculated following the equation (1) of Maguire (1962),

$$GRI = (N_i / D_i), \quad (1)$$

where N_i represents germinated seeds on the i^{th} day and D_i is the number of days from the commencement of the germination assay to the i^{th} day (see also Mijani *et al.* 2013). GRI was determined at six and 12 weeks for each treatment. Cumulative mean germination data (%) were plotted against time (weeks) from which the following indices were extracted: time-to-initiation of germination (T_1), time to 50% (T_{50}), and time to maximum germination (65% (T_{65})) in this study (see Soltani *et al.* 2002).

Estimation of Occurrence of Polyembryony

If only one radicle emerged during germination, seeds were considered mono-embryonic, but if two or more radicles were observed then the seeds were considered polyembryonic

(Firetti-Leggieri *et al.* 2013). Total percentage polyembryony was calculated from the proportion of seeds showing two or more radicles at germination for a given treatment. Seeds showing polyembryony were grouped into classes (twins, triplets and quadruplets) depending on the number of radicles that emerged during germination (Mendes-Rodrigues *et al.* 2012).

Confirmation of Polyembryony

Confirmation of polyembryony in the two forms of *D. unguis-cati* was further determined through seedling establishment (Firetti-Leggieri *et al.* 2013; Mendes-Rodrigues *et al.* 2012). After germination, polyembryonic siblings with at least one pair of leaves were taken out of the germination petri-dishes and transferred into 20 cm plastic pots filled with locally available commercial soil (Osmocote Multi-Purpose Potting Mix with trace elements). Seedlings were left to grow in a light environment (range: 60 – 250 $\mu mol.m^{-2}.s^{-1}$) over a 1-year period to ascertain if the individual seedlings would establish independent of other siblings, and whether each sibling would develop separate roots and tubers or not. The same process was repeated for polyembryonic seedlings in which the siblings were physically separated.

Data Analysis

Germination percentage data were arcsine transformed before analysis to improve normality of residuals. Analysis of variance (ANOVA) was used to test the effects of light, temperature regimes, and pod form of *D. unguis-cati*, as well as their interactions on germination indices and occurrence of polyembryony. The form of *D. unguis-cati*, light, and temperature regimes were used as fixed effects on the ANOVA model. A Tukey HSD post-hoc test was performed to assess the germination and polyembryony differences between the temperature treatments. When no significant interactions were detected, a Pearson's χ^2 statistical test was used to compare the frequency of polyembryonic seeds of LP and SP. All statistical tests were carried out at $\alpha < 0.05$ using the *R* statistical program on R version 3.1.0 (R Development Core Team 2014).

4.4 Results

Temperature and light effects on time-to-start and rate of germination

At all the temperature regimes, the seeds of the short pod (SP) form showed rapid germination whilst those of the long pod (LP) form were gradual and slower **(Table 4.1; Figure 4.1)**. Across the temperature range and photoperiod cycles tested, it took seeds of SP an average of 11.5 days to initiation of germination (T_1), except for the cool 10/20 °C regime where up to 28 days was required to T_1 **(Table 4.1)**. It took seeds of LP significantly longer period (an average of 20.2 days) to initiation of germination (T_1) across all temperatures, other than 10/20 °C in which no germination was recorded **(Figure 4.1a)**. The inhibitory effect of low temperatures (10/20 °C) on germination was more pronounced on seeds of the LP form than those of SP, since the former did not germinate at all at this temperature. When the experiment was terminated, seed germination in SP at 10/20 °C had reached about 42.9% **(Figure 4.1a)**. However, the gradient of the curve indicate that had the experiment continued, more seeds would have germinated with time, and therefore a higher total germination percentage could have been attained at this temperature too.

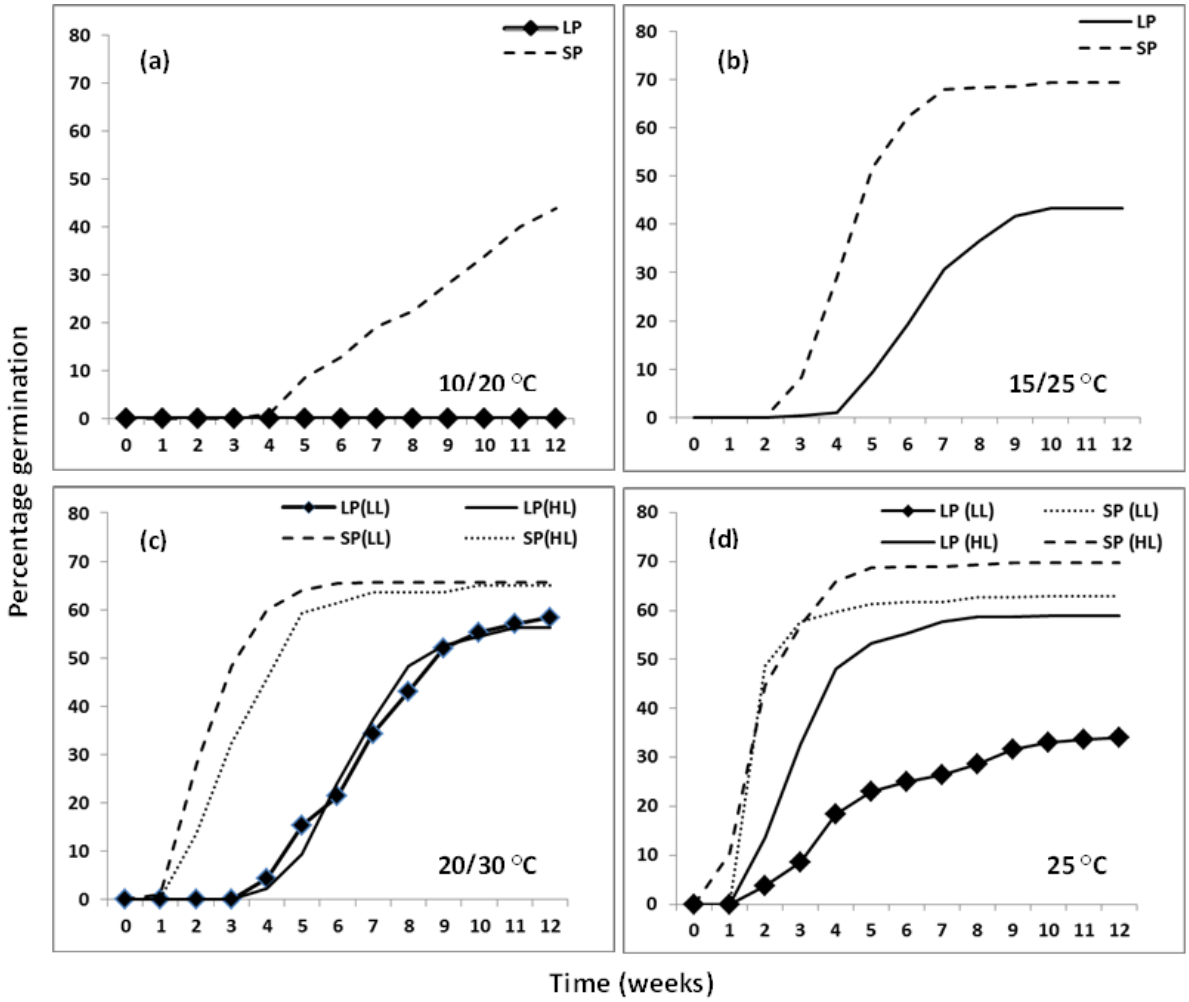


Figure 4.1. Cumulative mean germination percentage as a function of time for two forms of *D. unguis-cati* seeds at different regimes of 12 hour low/high temperature and photoperiod. LP: long pod;SP: short pod; LL: Low light = constant darkness; HL: High light conditions = 12 hour photoperiod. (a): 10/20 °C with constant darkness; (b): 15/25 °C with constant darkness; (c): 20/30 °C with both constant darkness and light conditions; (d): room temperature (25 °C) with both constant darkness and light conditions. Cumulative germination curves for the 30/45 °C temperature regime were not included because of insignificant germination incidents.

Time to 50% germination (T_{50}) was significantly lower for SP (range: 14 - 34 days) when compared to LP (range: 30 - 84 days) across the temperature regimes tested **(Table 4.1)**. Light did not have any significant effect on T_1, T_{50} or T_{65} for either LP or SP at 20/30 °C **(Figure 4.1c)**, but that was not the case at constant 25 °C for LP **(Figure 1d)**. Light had a significant germination effect on T_{50} on LP at 25 °C but not SP. At 25 ^{o}C, it took LP 30 days to reach T_{50} under light conditions but >84 days to reach T_{50} in constant darkness **(Table 4.1; Figure 4.1d)**. In contrast, at 25 ^{o}C, T_{50} for SP was 14 - 16 days, irrespective of the light conditions.

Table 4.1 Effect of temperature and light regimes on the time (days) to initiation of germination (T_1), time to 50% (T_{50}) and time to final (65% (T_{65})) germination for two forms of *D. unguis-cati*. These data were extracted from cumulative mean germination percentage curves plotted as a function of time. LL: Low light = 24 hour or constant darkness; HL: high light = 12 hour photoperiod; SP: short pod; LP: long pod. Data from the 30/45 °C temperature regime were not included in the table because germination incidents were minimal

	Time (days) to % germination					
	T_1		T_{50}		T_{65}	
Treatment	SP	LP	SP	LP	SP	LP
10/20 °C + LL	28	N/A	>84	N/A	>84	>84
15/25 °C + LL	21	28	34	>84	45	>84
20/30 °C + HL	7	28	30	58	70	>84
20/30 °C + LL	7	28	22	61	42	>84
25 °C + HL	3	10	16	30	27	>84
25 °C + LL	3	7	14	>84	77	>84
Mean response	11.5	20.2	>33.3	>63.4	>57.5	>84

Table 4.2 Summary results of ANOVA showing effects of the form of *D. unguis-cati*, temperature and light regimes on the germination rate index (GRI) and total germination %. Significant effects are shown in bold

Source of variation	df	GRI (6 weeks)		GRI (12 weeks)		Germination %	
		F-ratio	*P*-value	F-ratio	*P*-value	F-ratio	*P*-value
Form	1	174.148	<0.0001	102.664	<0.0001	82.604	<0.0001
Light	4	136.222	<0.0001	193.577	<0.0001	2.287	0.132
Temp	1	0.299	0.585	0.068	0.794	115.657	<0.0001
Form * Light	4	27.126	<0.0001	9.053	<0.0001	0.005	0.942
Form * Temp	1	5.164	0.024	2.368	0.125	9.732	<0.0001
Light * Temp	2	3.27	0.04	1.186	0.307	5.334	0.005
Form * Light * Temp	2	3.201	0.043	2.236	0.109	0.023	0.977
Error	218						
Total	234						

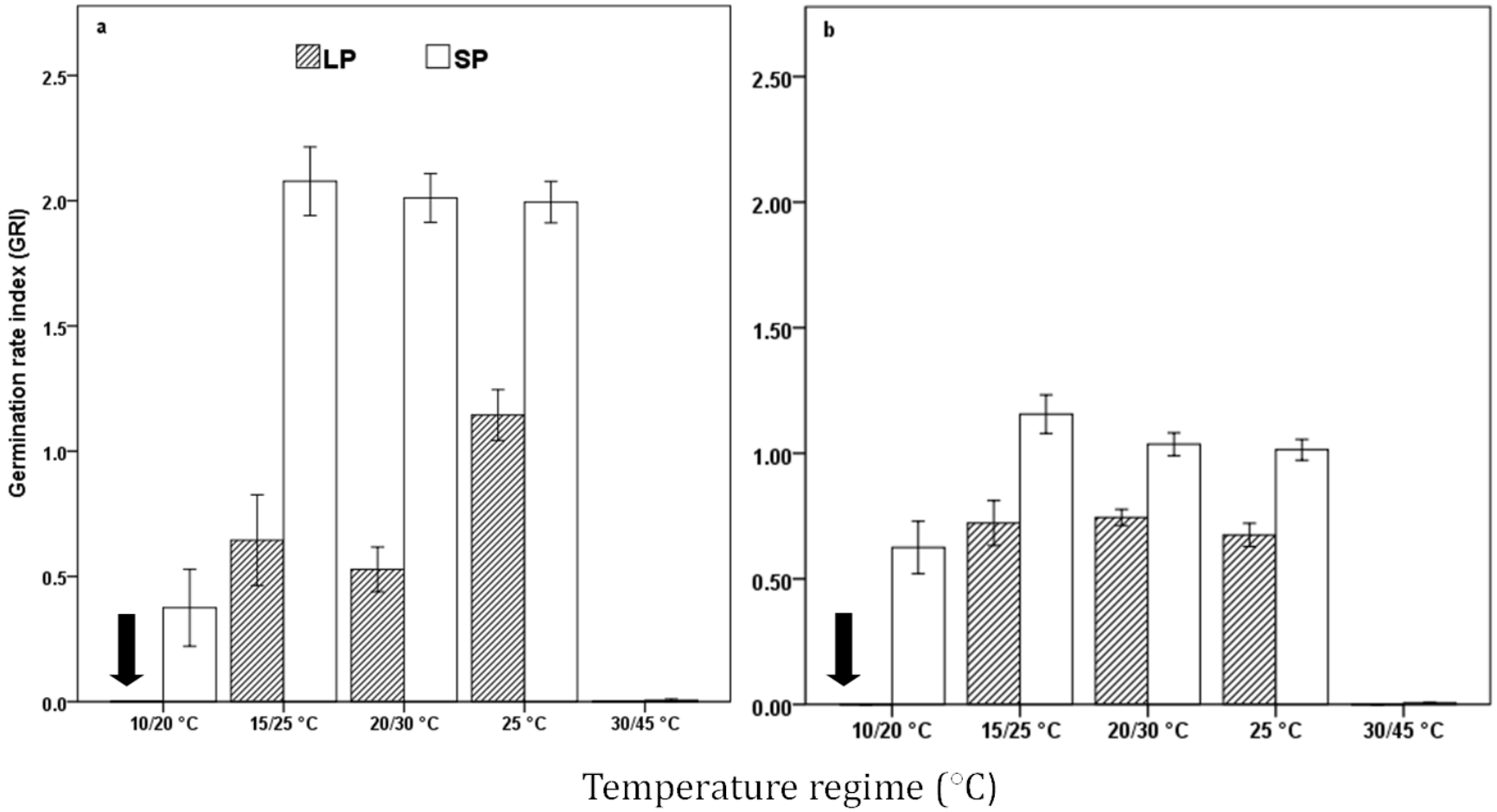


Figure 4.2. The effects of temperature on germination rate index (mean ± SE) for the two forms of *D. unguis-cati*, long pod (LP) and short pod (SP). (a) Germination rate index at 6-weeks since start of germination; (b) Germination rate index at the end of the germination assay, *i.e.*, 12 weeks since start of germination. Data for light and darkness levels were combined at each temperature regime because they were not significantly different. Arrows indicate no germination incidents for LP at 10/20 °C. The legend in (a) also applies in (b).

The rate (speed) of germination estimated by Maguire's germination rate index (GRI) showed significantly higher germination rates for SP than LP at all temperature regimes **(Figure 4.2).** The magnitude of the difference in germination rate between the two forms was also greater at 6-weeks compared to 12-weeks, especially for the moderate (15/25 °C) and warm (25 °C and 20/30 °C) temperature regimes **(Figure 4.2a, b; Table 4.2)**. At 6-weeks, GRI values for all interaction effects (pod form x light; pod form x temperature, light x temperature, and form x light x temperature) were significant ($P < 0.05$; **Table 4.2**), suggesting that GRI response values for seeds of each form varied significantly depending on light and/or temperature conditions. In contrast, at 12-weeks, only the interaction effect of form x light was significant ($F_{4, 218} = 9.05$; $P < 0.0001$) **(Table 4.2)**, implying a greater role of light than temperature regime on this germination index.

Occurrence and frequency of polyembryony

Polyembryony occurs in both forms of *D. unguis-cati* as demonstrated by emergence of two or more radicles from individual seeds during germination **(Figure 4.3a).** However, there was a significant difference in the frequency of polyembryony between LP and SP (χ^2=71.730, df=1, $p < 0.002$). SP displayed a significantly higher frequency of polyembryony than LP (SP 38.52 ± 2.74%; LP 4.68 ± 1.13%) (see **Figure 4.4** and **Figure 4.5)**.

Classes of polyembryony

Three classes (twin, triplets and quadruplets) of polyembryonic seedlings were observed in this study **(Figure 4.3)**. All classes were observed in SP: single (60.4%; N = 628/1040), twin (25.5%; N = 265/1040), triplet (11.7%; N = 122/1040) and quadruplet (2.01%; N = 21/1040) **(Figure 4.4)**. All polyembryonic seedlings in LP were twins, and constituted only 4.68% (N = 42/897) of germinated seeds **(Figure 4.4)**.

Seedling establishment of polyembryonic siblings

Polyembryonic seedlings were easily separated from each other, with each shoot system detaching with a corresponding radicle or root system **(Figure 4.6)**. Separated polyembryonic seedlings were able to develop and establish individually when transplanted into growth media and developed their own root system with tubers **(Figure 4.3c)**. When left intact and transferred

to growth media, polyembryonic siblings still developed individually with independent root and tuber systems. We also observed that for each set of polyembryonic siblings, one seedling was more robust and had a larger subterranean tuber than that of other siblings **(Figure 4.3)**.

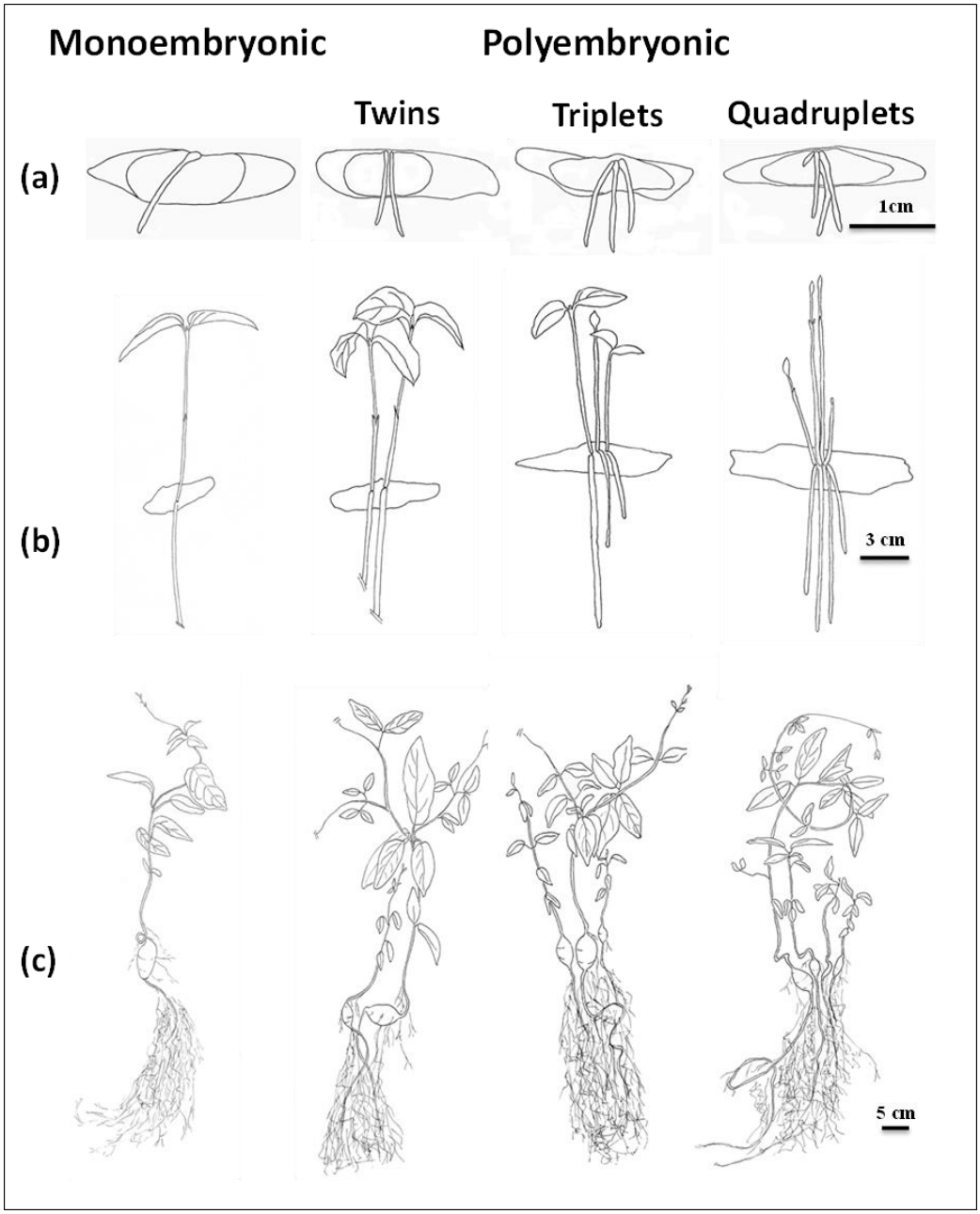


Figure 4.3. Illustrations of monoembryonic and polyembryonic seedlings of *D. unguis-cati* at different stages of development. Top row (a): Seeds showing emergence of radicles one week since start of germination. Middle row (b): Seedlings at 4-6 week since start of germination. Bottom row (c): One year old seedlings, with polyembryonic seedlings clearly showing independent development of tubers and root systems. (Illustrations by Tanya Scharaschkin).

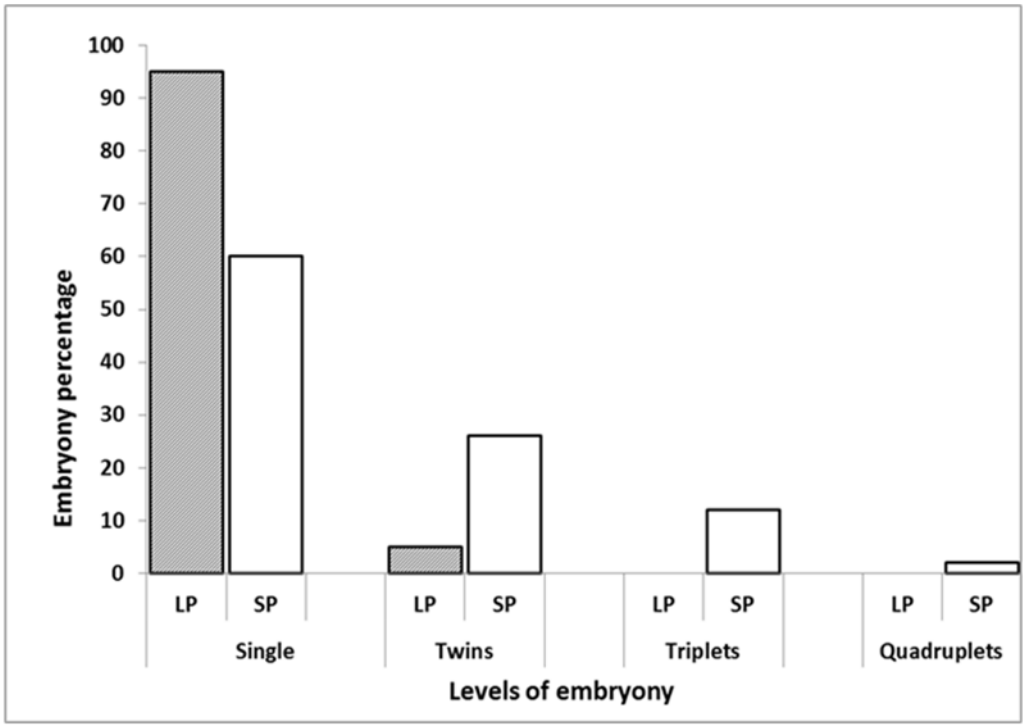


Figure 4.4. Frequency of different embryony classes in the two forms of *D. unguis-cati*, long pod (LP) and short pod (SP). Only twin seedlings were observed in LP polyembryonic seeds whereas SP also had triplet and quadruplet seedlings.

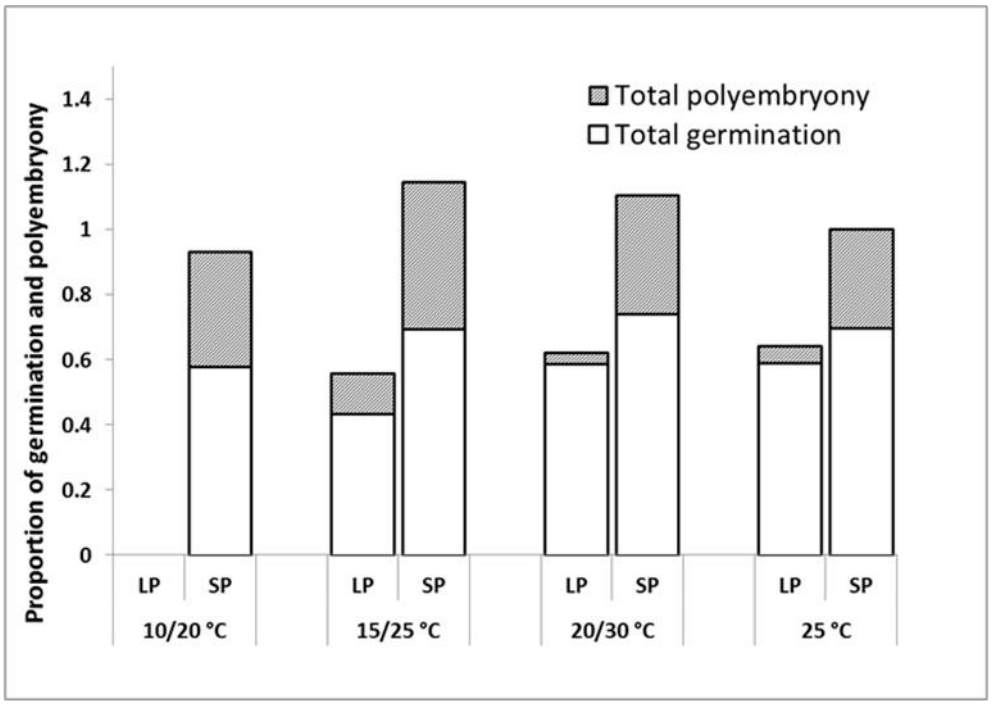


Figure 4.5. Comparison of the proportion of germination percentage (white) and polyembryony percentage (grey) of LP and SP for the same temperature regimes: 10/20°C, 15/25 °C, 20/30 °C and room temperature (25 °C). Differences in polyembryony frequency between temperature regimes were not significant within each form (F-value=0.902, df=1, p value= 0.345).

Polyembryony and Germination Rates of LP and SP

This study established that SP exhibited higher germination indices and higher frequency of polyembryony than LP, irrespective of temperature regimes **(Figure 4.5)**. There were no significant interaction effects of temperature and/or light regime on the occurrence, frequency or classes of polyembryony (temperature x plant form: F-ratio = 0.594, df=1, p = 0.443; light x plant form: F-ratio = 0.021, df = 1, p = 0.886), implying that these environmental resources did not influence the dynamics of polyembryony. Nonetheless, both LP and SP showed their highest frequency of polyembryony (15.9 and 41.2%, respectively) at 15/25 °C **(Figure 4.5)**. At 30/45 °C, only a few incidents (0.3 %) of germination and no polyembryony was observed for SP and no germination whatsoever for LP.

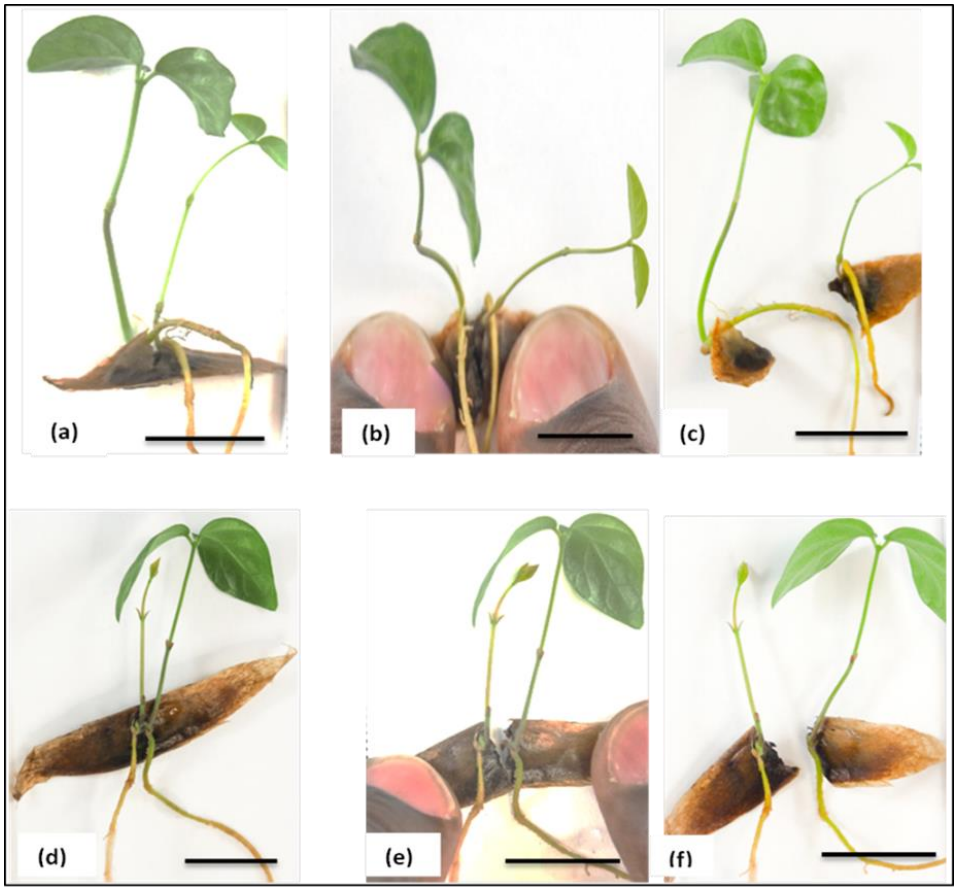


Figure 4.6. Multiple seedlings emerging from a single seed of *D. unguis-cati* four weeks since start of germination; a-c: short pod (SP) and d-f: long pod (LP). Intact seeds showing emergence of twin seedlings (a, d); (Partial separation of seeds into two halves to separate the two seedlings from the same seed (b, e); Complete separation of seedlings emerging from the same polyembryonic seed (c, f). Scale bars represent 1 cm.

4.5 Discussion

Our results indicate that, on average, there are significant differences in the seed germination responses of the two forms of the invasive woody vine, *D. unguis-cati*, under varying temperature and light regimes. Seeds of SP exhibit higher mean value as well greater variation in germination niche (from cool to warm temperatures) than those of LP (warm temperatures only) **(Figures 4.1** and **4.2)**. The polyembryony results confirm previous findings by (Vivian-Smith and Panetta 2004b) of occurrence of multiple seedlings from single seeds in SP. However, the two forms exhibit different frequencies of polyembryony: about 40% in SP and a much lower frequency (often <5%) in LP.

Predicting invasiveness potential of the two forms of D. unguis-cati

Our results suggest that germination niche requirements of SP are broad and non-specific, while LP germinates optimally only under warmer temperature conditions (20/30 °C and 25 °C; **Figure 4.1; Table 4.1)**. Flexible germination cues enhance the invasive capacity of plants by enabling them to spread and establish in novel climatic conditions of recipient communities (Dechoum *et al.* 2015b; Wainwright and Cleland 2013). SP had a much higher germination rate (compared to LP) at the cooler temperature regime of 10/20 °C. Equally, although of low frequency, SP showed evidence of germination incidents at hot, 30/45 °C temperature regime while there was no germination at all for LP under this scenario. These wider germination amplitudes may indicate greater resilience in SP seeds, and may suggest a potential for this form of *D. unguis-cati* to spread further into the cooler state of New South Wales and Victoria as well as into the warmer/hotter areas of Australia (e.g., Northern Territory and western Queensland), especially under a climate change scenario. In general, the rapid germination behaviour of SP seeds SP **(Figure 4.1)** is typical of invasive species (Ferreras *et al.* 2015; Wainwright and Cleland 2013). Whilst the longevity of SP seeds is low (<12% by 1 year) (Vivian-Smith and Panetta 2004b), its rapid germination under a wider range of temperatures may confer a fitness advantage in terms of seedling establishment and spread.

The higher frequency of polyembryony in SP could also allow it to proliferate successfully in a variable environment in contrast with LP **(Figures 4.4** and **4.5)**. Thus for the same number of initial seeds introduced in a new environment, there is a greater likelihood that more SP, rather than LP, plants would establish. Considering that twins, triplets and quadruplets occur in

polyembryonic SP seeds, while only twins occur in LP (and at a lower frequency), it can be assumed that a higher propagule pressure would be exerted by SP than LP upon introduction. Polyembryony may also increase invasiveness potentials by the bet-hedging strategy, which ensures that at least one individual seedling from a polyembryonic seed survives (Hotchkiss *et al.* 2008; Ladd and Cappuccino 2005a). Although mature LP fruits are known to have twice as many seeds per fruit as SP fruits (Shortus and Dhileepan 2011), higher germination and polyembryony exhibited by SP may likely increase propagule pressure leading to SP being the more invasive form than LP. Propagule pressure has previously been correlated with plant invasiveness (Moravcová *et al.* 2015; Pyšek and Richardson 2007; van Kleunen *et al.* 2015). Nonetheless, caution is needed in the interpretation of this finding because the polyembryony phenomenon may not necessarily be adaptive as it puts siblings into direct competition for environmental resources (Blanchard *et al.* 2010; Hotchkiss *et al.* 2008).

Some evidence suggests that polyembryony reduces seed germinability significantly (Mendes-Rodrigues *et al.* 2012), but our results do not support this position. SP consistently had higher germination rates than LP at all temperature regimes whilst also exhibiting higher frequency of polyembryony than LP. Temperature did not significantly affect expression of polyembryony, suggesting a genetic basis for the phenomenon (Batygina and Vinogradova 2007), but both LP and SP appeared to show relatively higher polyembryony frequencies at 15/25 °C (their optimal growth condition) than at other temperature regimes. Our preliminary observations of 1-year old siblings from polyembryonic seeds indicate equal survival rates, but slower mean growth rates per individual as the number of siblings increase **(Figure 4.3;** JC Buru, unpublished data**)**. However, it remains to be seen how differences in germination and polyembryony rates will translate to initial biomass gain per individual, and ultimately offspring fitness.

The differences in the invaded range distributions of the two forms could be a product of colonization events, with the LP form being a more recent arrival in Australia compared to the SP form. There may also be an underlying genetic basis responsible for the observed pattern (Hollister 2015). Although largely untested, and therefore speculative, the prevalence of SP in Australia may potentially be a classic case of (i) recent whole genome duplication followed by diploidization during which genes are lost/modified/rearranged (Hollister *et al.* 2012; Otto and Whitton 2000), and/or (ii) release from natural enemies in the novel environment. Both scenarios

have the tendency to increase competitiveness, niche pre-emption, and ultimately spread and distribution (Elton 1958; Keane and Crawley 2002; Mitchell and Power 2003).

LP and SP: Are They The Same Species?

The differences in germination dynamics and frequency of polyembryony between SP and LP lends further credence to suggestions that the two forms of *D. unguis-cati* could be two extremes of the same species or even different species (Boyne *et al.* 2013a). Anecdotal evidence suggests that the predominant form of *D. unguis-cati* in the native range is similar in appearance to the form being referred to as LP in Australia (Dhileepan K. personal comm.). Interestingly, an earlier study conducted within the native range found that *D. unguis-cati* did not exhibit any polyembryony (Firetti-Leggieri *et al.* 2013). This is in sharp contrast to what we have observed in SP (40%), but is closer to our results for LP (<5%). Whether the two forms are different species, products of two independent introductions from the native range of the weed, or arose from (auto-/allo-) polyploidy remains to be determined. Only a comprehensive phylogenetic study of the different forms of the species and other members of the genus *Dolichandra* will help clarify the status of the two forms (but see Prentis *et al.* 2009). Moreover, work on chromosome number (karyotype), level of polyploidy (a trait known to correlate significantly with polyembryony (Firetti-Leggieri *et al.* 2013; Hollister 2015), as well understanding the interplay of their breeding systems (sexual vs. asexual) variation (Osunkoya *et al.* 2009), will also help our understanding of their invasiveness potential.

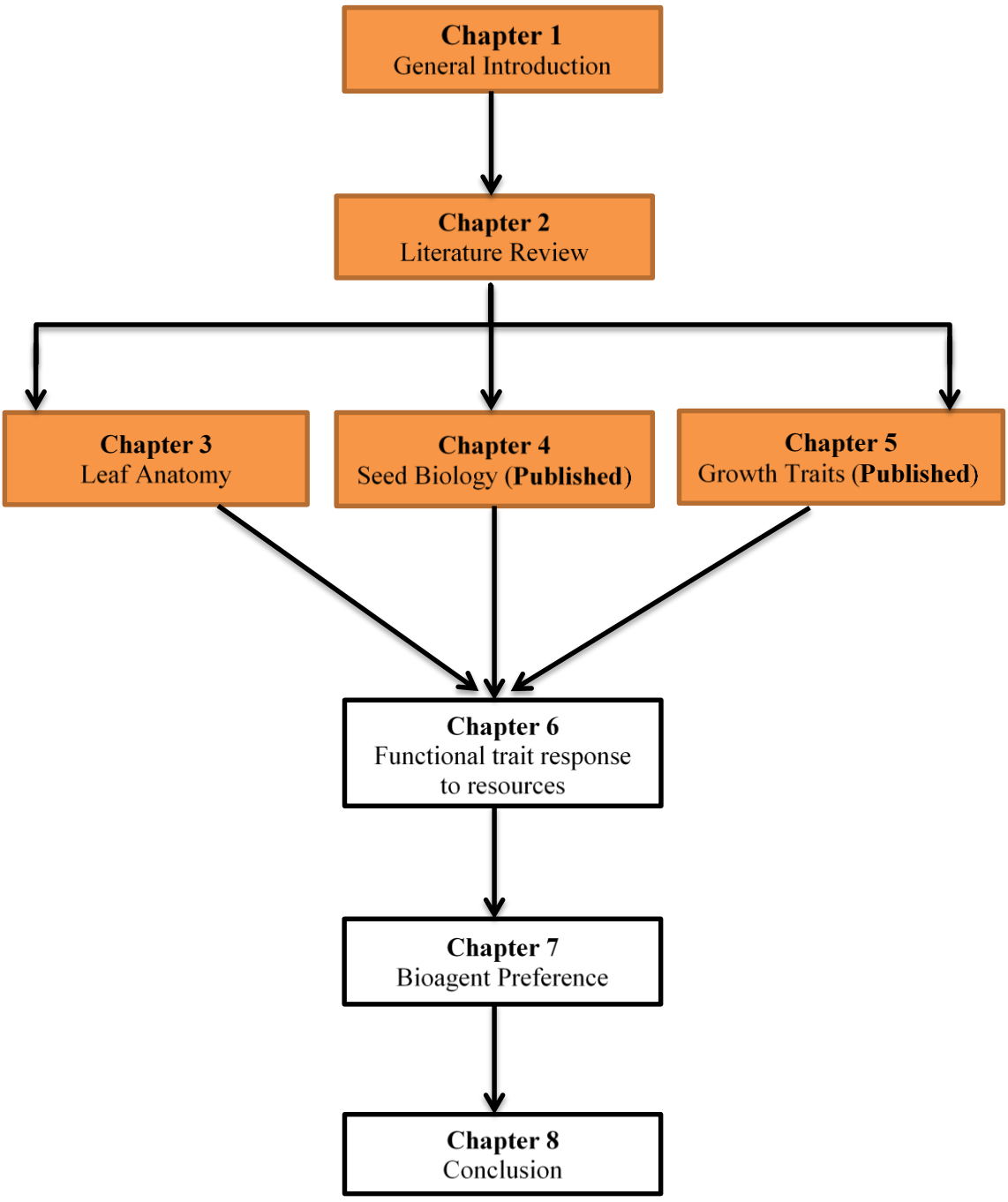
4.6 Conclusions and Recommendations

The current study is the first to report on the comparative germination rates of the two forms of *D. unguis-cati* in Australia. Further germination assays could involve other conditions such as different moisture thresholds and seed burial and retrieval experiments to explore the extent and contribution of above environmental cues to variation in invasiveness of the two forms. To ascertain the ecological consequence (fitness) of polyembryony on *D. unguis-cati*, future studies should consider comparing growth rates of mono- and poly-embryonic seedlings in intra- and inter-specific competition. Due to the vast differences in the germination behaviour, floral (Dhileepan *et al.* 2013) and leaf morphological/physiological traits of the two forms of *D. unguis-cati* (Boyne *et al.* 2013a), different control strategies should be considered for these two

forms. Currently, the same chemical and biological control strategies are used to manage the two forms, thus potentially compromising the efficacy of these control options. Leaf-feeding biocontrol agents have been released (Dhileepan *et al.* 2007a; Dhileepan *et al.* 2013; Dhileepan *et al.* 2010), and they are applied to both LP and SP forms. However, whether these agents are equally effective in controlling both forms is yet to be determined. The need for additional fruit- or seed-eating biocontrol agents has been previously highlighted (Osunkoya *et al.* 2009) and is further supported by the current findings, in which we have demonstrated potential roles of propagule pressure and polyembryony as drivers of spread of *D. unguis-cati*. The efficacy of fruit- or seed-attacking agents as a biocontrol strategy should be considered for both forms of the weed, but perhaps more so for SP, given the results of this study. It will be well worth investigating if the same germination dynamics and frequency of polyembryony are observed in *D. unguis-cati* in other parts of its native and invaded ranges, as it could shed light on the role played by propagule pressure in the spread of weeds.

Acknowledgements

We acknowledge technical assistance provided by Mark Crase and Amy Carmichael (QUT) and thank Liz Snow of the Department of Agriculture and Fisheries (DAF) in Brisbane for helping with seed collection. Thanks to the Plant Structure and Systematics Research Group at QUT and Dr. Jennifer Firn for providing valuable feedback on an earlier version of the manuscript. We thank two anonymous reviewers for constructive comments on an earlier version of this manuscript. The first author was funded by a scholarship from the Government of Botswana and DAF (Queensland, Australia) while performing this research**.**



Statement of Contribution: The first author was responsible for conducting the experiments, data collection, data analysis and interpretation, and writing the manuscript. The co-authors were involved in refining the methodology, providing statistical support during data analysis and editing of the manuscript. Co-authors also provided logistical support during the experiments

Chapter 5: Comparison of growth traits between abundant and uncommon forms of a non-native vine, *Dolichandra unguis-cati* (Bignoniaceae) in Australia

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5.1 Abstract

Cat's claw creeper vine, *Dolichandra unguis-cati* (L.) Lohmann (syn. *Macfadyena unguis-cati* (L.) Gentry) (Bignoniaceae), is a major environmental weed in Australia. Two distinct forms of this weed ('long' and 'short' pod), with differences in leaf morphology and fruit size, occur in Australia. The long pod form has only been reported in less than fifteen localities in the whole of south-east Queensland, while the short pod form is widely distributed in Queensland and New South Wales. This study sought to compare growth traits such as specific leaf area, relative growth rate, stem length, shoot/root ratio, tuber biomass and branching architecture between these forms. These traits were monitored under glasshouse conditions over a period of 18 months. Short pod exhibited higher values of relative growth rates, stem length, number of tubers and specific leaf area than long pod, but only after 10 months of plant growth. Prior to this, long and short pod did not differ significantly. Higher values for these traits have been described as characteristics of successful colonizers. Results from this study could partly explain why the

short pod form is more widely distributed in Australia while long pod is confined to a few localities.

Keywords: Cat's claw creeper, invasive species, competitiveness, relative growth rate, successful colonizers, traits, biomass, tubers

5.2 Introduction

Invasive plant species continue to threaten biodiversity and ecosystem function globally (Heckel 2004; Pimentel *et al.* 2005). A fundamental objective of invasion ecology is to identify a suite of plant traits that may determine invasion success in novel environments (Pyšek and Richardson 2007; Richardson and Pyšek 2006; van Kleunen *et al.* 2010b). An outcome of this search can be traced back to Baker's ideal weed hypothesis, in which Baker (1965) proposed a set of plant traits most likely to be exhibited by invasive species . Comparative studies between exotic invasive species and their native non-invasive congeners have contributed immensely to our understanding of traits that promote colonisation and invasion success by some species (e.g. van Kleunen *et al.* 2011).

It has proven difficult to consistently find a correlation of the same set of traits with invasiveness, likely because of the varying effects of environmental factors on different plant species (Alpert *et al.* 2000; Burns 2006). Studies have shown that no particular trait solely confers invasiveness on a species, rather it is how a species responds to different environmental conditions that contributes to its fitness and abundance (Firn *et al.* 2012; Leishman *et al.* 2010; Osunkoya *et al.* 2010b; Pattison *et al.* 1998a). Plastic responses of invasive plants to varying environmental conditions increase their competitiveness and fitness (Claridge and Franklin 2002a). Therefore, multiple factors likely explain the success of invasive plant species (Blumenthal 2005; Daehler 2003; Lamarque *et al.* 2011; Leffler *et al.* 2014; Leung *et al.* 2004; MacDougall *et al.* 2009). For example, Burns (2006) found that invasive species had higher specific leaf area (SLA) and relative growth rates (RGR), but only under certain environmental conditions. Nevertheless, a pattern of relatedness to invasiveness has been reported for some plant traits (Pyšek and Richardson 2007). Mostly, traits that have direct relatedness to plant physiological performance such as leaf area ratio, growth rate, shoot/root allocation and

propagule pressure show marked differences between evidently invasive and non-invasive species (Grotkopp *et al.* 2002; van Kleunen *et al.* 2010b).

Invasive species were shown to have higher values of traits like SLA (Burns 2006; Lake and Leishman 2004), RGR (Dawson *et al.* 2011), and more biomass allocated to organs like stems, resulting in taller plants (Gallagher *et al.* 2015; Stanisci *et al.* 2010; van Kleunen *et al.* 2015). High SLA is often associated with high RGR (Grotkopp and Rejmánek 2007), although other studies have not found that trend (see, for example, Garcia-Serrano *et al.* 2005). Overall, fast growing plants have generally been found to be more likely to be invasive than others (Blumenthal and Hufbauer 2007; Lake and Leishman 2004; Richardson 1998). Higher values for these traits in invasive species compared to less invasive ones imply different strategies for capturing and using resources such as light, carbon, nitrogen and moisture (Gallagher *et al.* 2015). Because resources are almost always limiting in the environment (Cordell *et al.* 1998), efficient use of limiting resources by invasive species can enhance their colonizing success (Pattison *et al.* 1998a). In disturbed environments, species that are better able to exploit fluctuating resources will likely invade the system (Cordell *et al.* 1998; Leffler *et al.* 2014; van Kleunen *et al.* 2010b).

Most studies aimed at understanding differences in traits associated with invasion success have used native species as control plants (Muth and Pigliucci 2006). The limitation of this approach is that these native species may already be invasive elsewhere (van Kleunen *et al.* 2010b). For example, some native species used in a comparative study by Godoy *et al.* (2011) were reported to be invasive in other parts of the world. Other studies have also shown that these traits do not always differ between invasive and non-invasive species (Meiners 2007; Smith and Knapp 2001; Thompson *et al.* 1995). An assessment of 122 species including non-native invasive and native species that occupy disturbed areas did not find significant differences in these traits (Leishman *et al.* 2010). Muth and Pigliucci (2006) argue that some native species were shown to have invasive tendencies in their native range, implying that introduced vs native species comparisons may not always be informative (but see Blossey and Notzold 1995; Callaway and Ridenour 2004; Dawson *et al.* 2015b; Keane and Crawley 2002; van Kleunen *et al.* 2011). There could also be a bias in choosing highly competitive invasive species and comparing them with known weak native competitors in pairwise experiments (Vila and Weiner 2004) or comparing phylogenetically non-related species (Burns 2006).

Our understanding of invasiveness traits could be better enhanced by comparing related non-native species of varying levels of colonization success (Kolar and Lodge 2001; Muth and Pigliucci 2006; van Kleunen *et al.* 2010b). In this study, we compare different traits between two forms of an invasive vine, cat's claw creeper, that appear to have significantly different levels of invasion success. Cat's claw creeper, *Dolichandra unguis-cati* (L.) Lohmann (syn. *Macfadyena unguis-cati* (L.) Gentry) was introduced as an ornamental into Australia from South America in the 1800s (Dhileepan 2012; Downey and Turnbull 2007; Gentry 1976). *D. unguis-cati* is now a declared environmental weed and considered formally as a Weed of National Significance (WoNS) in Australia (Thorp and Lynch 2000).

Dolichandra unguis-cati prefers forested and riparian habitats, although it also grows vigorously on dry road side sunny environments. It also appears to thrive in most soil types, tolerating a wide range of soil pH (Downey and Turnbull 2007). Two forms of this species with distinct leaf morphology occur in Australia (Dhileepan 2012; Shortus and Dhileepan 2011). The two forms of *D. unguis-cati* were named long pod (LP) and short pod (SP) due to differences in their average fruit (pod) length at maturity (LP: 700.2 ± 23.5 mm; SP: 300.9 ± 89.6 mm) (Shortus and Dhileepan 2011). While LP occurs in isolated localities of south-east Queensland (Qld), SP occurs extensively in Qld and New South Wales, often in dense infestations (Dhileepan 2012; Downey and Turnbull 2007). These two forms appear to prefer similar habitats, although there is general lack of research on the ecology of this species (Osunkoya *et al.* 2009). The LP and SP forms have been shown to carry an average of 120 ± 10 and 60 ± 23 seeds per pod at maturity, respectively (Shortus and Dhileepan 2011). Seeds of both forms are two-winged, papery and flattened/oblong in shape, 10 - 18 mm long, 4.2 - 5.8 mm wide. The average seed biomass is not significantly different between the forms of *D. unguis-cati* (mean seed biomass for LP: 16.60 ± 0.65 mg and for SP: 15.65 ± 0.83 mg) (Shortus and Dhileepan 2011). Previous studies have found that the two forms showed differences in some life history traits. Boyne *et al.* (2013a) found a wide variety of leaf morphology for this species, but also reported that SP had significantly more simple leaves than LP.

In a field experiment using plants generated from tuberlings, Taylor and Dhileepan (2012) found that LP produced greater total dry mass (hence higher RGR) than SP although the study did not measure such parameters as specific leaf area (SLA) and leaf area ratio (LAR). SP was shown to have rapid and higher germination rates than LP at varying temperature regimes (Buru *et al.* 2014). SP was also reported to exhibit significantly higher frequencies of polyembryony

than LP, at times one seed producing quadruplet seedlings (Buru *et al.* 2016). The only study on the seed bank ecology of the most prevalent form (SP) by Vivian-Smith and Panetta (2004b), found it to have low seed longevity, usually less than 12 and 1% at 1 year for soil-surface (< 1 cm depth) and 5 cm depth buried seeds, respectively. Osunkoya *et al.* (2009) also noted some differences in stem density of genets and ramets between the two forms in field samples, but decried lack of data on growth rates and reproductive capacity for the two forms.

Herbarium records and field surveys suggest that LP is widely distributed in the native range, occurring from Mexico, Nicaragua, Costa Rica, Columbia to Brazil, whereas SP appear to be restricted to Paraguay (Dhileepan 2012; K. Dhileepan, personal observations). In Australia, previous field surveys have revealed that there were seven sites in south-east Queensland (Qld) where LP has been reported, two at which it co-occurs with SP (Boyne *et al.* 2013a; Dhileepan 2012; Shortus and Dhileepan 2011). Recently, seven more sites were identified, bringing the total number of known sites to 14 in south-east Qld where LP occurs (Liz Snow (Biosecurity Queensland), pers. Comm. 7/03/2016).

The cause for the observed differences in abundance levels between LP and SP is not yet established, but introduction pressure may be one explanation. Reconstructing the invasion history of this exotic species (or the two forms) is not possible because there are no records of their introduction, except that the species was first reported in a Melbourne Nursery catalogue in 1865 (Downey and Turnbull 2007). Introduction history of most ornamental plants is generally not or poorly recorded (Harris *et al.* 2007; Prentis *et al.* 2009). Studies on whether there has been any deliberate breeding selection of the species that resulted in the two forms are yet to be done.

Another explanation could be differences in growth strategies between LP and SP. Rapid growth and efficient resource allocation enhance success in colonization, especially during the early stages of plant life history (Bachmann *et al.* 2012; Luo *et al.* 2015). Considering that LP and SP show marked abundance differences in Australia, comparing important functional traits of the two forms may assist with understanding whether different growth strategies explain the different populations. Significantly higher values of growth related traits for one form could infer different strategies of resource use (Dawson *et al.* 2011; Godoy *et al.* 2011). Here we sought to compare traits such as SLA, RGR, stem length, shoot/root ratio, tuber biomass and branching architecture between the two forms of *D. unguis-cati* plants grown from seeds. We did this to develop a type of prospectus on the growing strategies of the two forms of *D. unguis-cati* that may begin to explain differences in their distributions and abundance.

5.3 Materials and Methods

Experimental design

In 2013 seeds of LP and SP were collected from various sites around the greater Brisbane area in southeast Queensland, Australia. Sites were chosen based on accessibility and availability of mature seeds at the time of experimentation. Once collected, seeds were stored for two weeks at room temperature in paper envelopes that were placed in containers with silica gel to ensure they were dry before germination commenced. Seeds were sterilised by soaking in 1% sodium hypochlorite (NaOCl) for 5 minutes followed by rinsing in water for 3 minutes (Mijani *et al.* 2013). Seed germination dynamics of the two forms carried out in growth chambers were discussed in detail in Buru *et al.* (2016) (Chapter 4).

After two weeks of germination, seedlings were transferred into plastic pots (dimensions: Width=200 mm, Height=190 mm, Length=200 mm) filled with locally available commercial multi-purpose potting mix (Osmocote) containing a professional wetting agent and trace elements. This seedling growth experiment was set up at the Ecosciences Precinct glasshouse facilities (GPS coordinates: 27°29'41.5248'' S; 153°1'49.2132" E) in Brisbane, Australia. The average temperature during the warmer months (October – April) ranged from 18 °C to 35 °C while during the cooler months (May – September) it was between 10 °C and 23 °C. Relative humidity ranged between 50 – 60% during this study. Plants were watered once a day but no additional fertilizer/nutrients were added. For this experiment, plants were left to grow without any support. Seedlings were left to grow in a light environment (range: 60 – 250 $\mu mol.m^{-2}.s^{-1}$) over 18 months (October 2013-March 2015), with sub-samples of plants taken at 5 and 10 months. Seven seedlings (replicates) were used per form (LP and SP) at each observation time. These replicates were randomly selected from an initial pool of over 100 plants raised from seeds. The remaining plants were used for other eco-physiological studies.

At observation time, vernier callipers were used to measure basal stem diameter (BSD) at the root-stem junction. Leaf area was determined by taking leaf pictures against a graduated background using a Panasonic DMC-ZS7, Lumix camera and then using the open access software Image J 1.47v (www.imagej.nih.gov/ij) to calculate the leaf area in cm^2. Two mature leaves (including petiole) per replicate were used for this purpose. Fresh and dry masses of these leaves were also determined.

For each replicate plant, stem length, number of primary branches and ramifications (secondary branches), number of tubers and tuber fresh weight were also recorded. Apical dominance index (ADI) was calculated by dividing the number of ramifications by the total length of the branch in metres according to Pérez-Harguindeguy *et al.* (2013). At each harvest period, whole plants were separated into above- and below-ground parts. Shoots, roots and tubers were separated and then dried in an oven at 80 °C for 72 hours (Cornelissen *et al.* 2003). Dry weights were measured using an electronic analytical model AUW120D, Mettler Toledo digital scale. Root, shoot and tuber dry weights were divided by the total dry weight to determine root, shoot and tuber mass ratio respectively (Garcia-Serrano *et al.* 2005). RGR was estimated by absolute change in total dry weight, above- and below-ground tissue dry weight, tuber dry weight and stem length between the 10^{th} and 18^{th} month divided by the number of months (see Taylor and Dhileepan 2012). Other resultant parameters such as specific leaf area (SLA) and leaf dry matter content (LDMC) or leaf matter per area (LMA) were calculated following Cornelissen *et al.* (2003) and Pérez-Harguindeguy *et al.* (2013).

Statistical analysis

Differences in RGR and other traits such as SLA, LDMC, total dry mass, belowground/aboveground biomass ratio, number of tubers, tuber mass ratio (TMR), shoot mass ratio (SMR) and root mass ratio (RMR) were compared using two-way MANOVA model, with form and age of plant as independent variables. Interactions of form and age of plants were also included in the model. When significant differences were found, a Tukey LSD post-hoc test was performed to check differences between specific means. Differences or similarities in plant traits between LP and SP were further analysed using a Principal Component Analysis (PCA). The clusters were projected on the graphical representation of the first two PCA axes. All statistical tests were conducted using R version 3.1.0 (R Development Core Team 2014). PCA was performed using an add-on *vegan* package (version 2.3-4) in R (Dixon 2003).

5.4 Results

Biomass production and allocation

The overall total dry mass differed significantly between the two forms after 18 months of plant growth ($F_{1, 36} = 73.802$, $p < 0.001$). There was a significant interaction between form and age of the plant on the total dry mass ($F_{2, 36} = 6.371$, $p < 0.004$). During the earlier stages of growth up to 10 months, there was no significant difference between the two forms in terms of total dry mass accumulation, although generally SP weighed more **(Table 5.1 and Figure 5.1a).**

Above- and below-ground biomass allocation (also shown by shoot/root ratio) did not vary significantly between forms ($F_{1, 39} = 2.568$, $p > 0.08$), and no significant interactions of form and age of plant were detected on this trait. A Tukey test of multiple comparisons of means showed that the proportion of dry biomass allocated to shoots, roots and tubers differed significantly between LP and SP after 18 months of plant growth ($P < 0.0005$, 0.021 and 0.002, respectively). SP allocated more biomass to tubers, shoots (leaves + stems) and roots than LP, especially after 18 months of growth **(Figure 5.1b).**

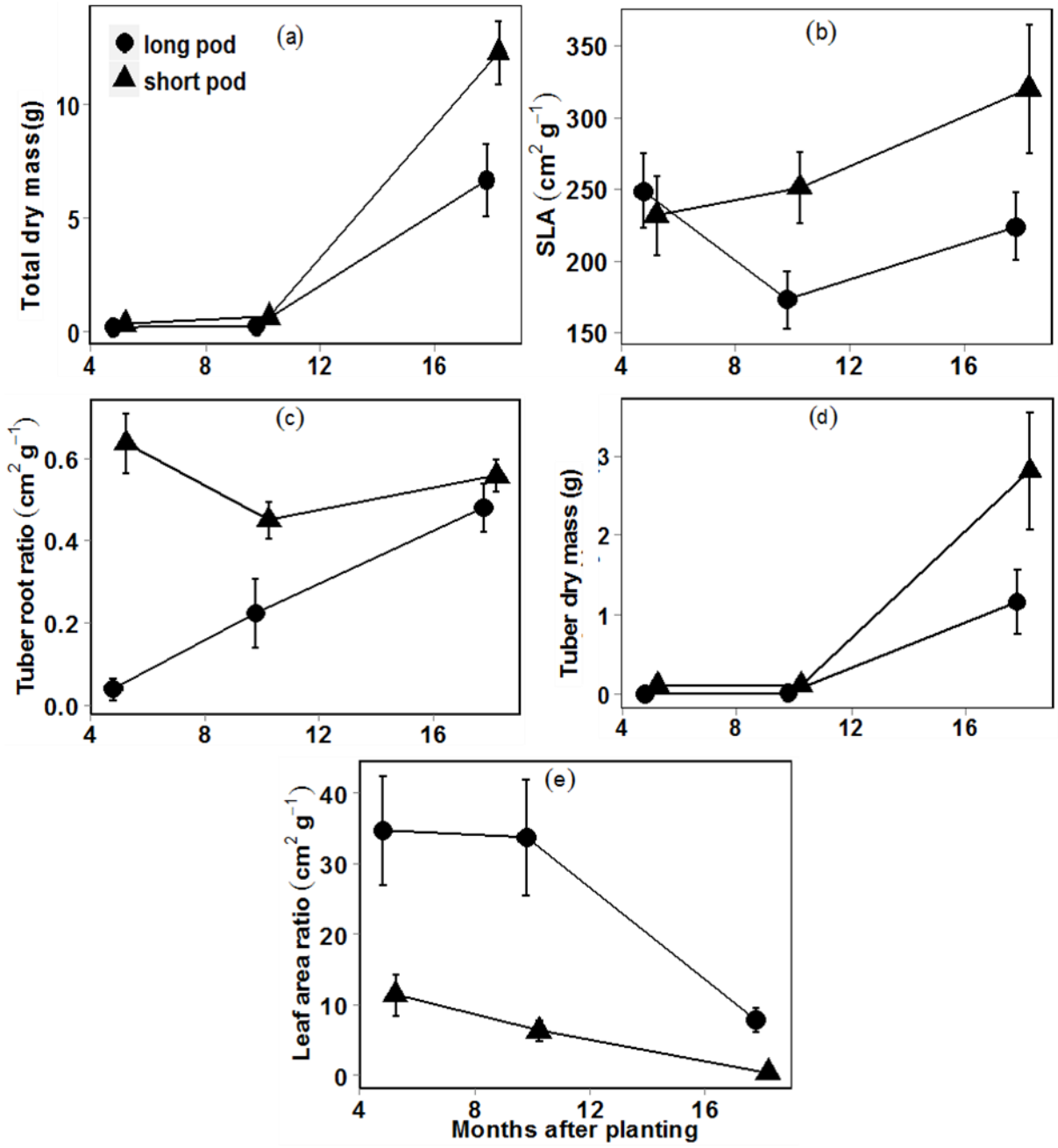


Figure 5.1. Total biomass production and allocation patterns (± SE; N=7) to tubers and leaves for long pod and short pod over time. (a) Total dry mass; (b) Specific leaf area (SLA); (c) Tuber/root ratio; (d) Tuber dry mass and (e) leaf area ratio. The legend in panel (a) applies to the rest of the panels.

Table 5.1 Mean (± SE) growth traits calculated at 5, 10 and 18 months after planting for LP and SP. Different letters indicate significant differences among age groups and between the two forms of *Dolichandra unguis-cati*. Summary ANOVA refers to F- and P-values of a MANOVA model of growth traits using fixed effects of form and age of plants, and an interaction of form: age of plants; d.f = 5, 36. Within each row, representing means across the age of plants, means with the same subscripts are not significantly different at $\alpha \leq 0.05$ using a Tukey LSD multiple comparison procedure. "***"= P≤0.0001; "**" = P≤0.001; "*" = P≤0.05; n.s = not significant

Traits	Age of plants in months						Summary ANOVA	
	5		10		18			
	LP	SP	LP	SP	LP	SP	*F*-ratio	Signif.
Aboveground dry mass (g)	$0.099_{a} \pm 0.023$	$0.200_{a} \pm 0.021$	$0.201_{a} \pm 0.047$	$0.430_{a} \pm 0.081$	$4.460_{b} \pm 0.922$	$7.580_{c} \pm 0.677$	6.968	*
Root dry mass (g)	$0.097_{a} \pm 0.023$	$0.057_{ab} \pm 0.009$	$0.073_{ab} \pm 0.019$	$0.137_{ab} \pm 0.027$	$1.043_{b} \pm 0.328$	$1.903_{c} \pm 0.295$	5.524	*
Root mass ratio (RMR)	$0.512_{a} \pm 0.062$	$0.151_{b} \pm 0.018$	$0.245_{c} \pm 0.035$	$0.221_{c} \pm 0.043$	$0.151_{b} \pm 0.016$	$0.154_{b} \pm 0.015$	17.990	***
Belowground dry mass (g)	$0.101_{a} \pm 0.029$	$0.174_{a} \pm 0.034$	$0.089_{b} \pm 0.018$	$0.253_{c} \pm 0.030$	$2.211_{c} \pm 0.723$	$4.719_{d} \pm 1.019$	5.440	*
Tuber dry mass (g)	$0.004_{a} \pm 0.003$	$0.117_{b} \pm 0.028$	$0.016_{c} \pm 0.007$	$0.118_{b} \pm 0.024$	$1.169_{d} \pm 0.412$	$2.816_{d} \pm 0.745$	4.923	*
Tuber mass ratio (TMR)	$0.020_{a} \pm 0.015$	$0.303_{b} \pm 0.053$	$0.071_{a} \pm 0.029$	$0.170_{c} \pm 0.020$	$0.148_{c} \pm 0.026$	$0.210_{d} \pm 0.038$	9.163	**
Total dry mass (g)	$0.200_{a} \pm 0.039$	$0.374_{a} \pm 0.034$	$0.290_{a} \pm 0.061$	$0.683_{b} \pm 0.116$	$6.671_{c} \pm 1.591$	$12.299_{d} \pm 1.391$	7.455	**
Shoot/root ratio (SRR)	$1.100_{a} \pm 0.270$	$4.556_{b} \pm 1.439$	$3.367_{c} \pm 0.726$	$3.540_{c} \pm 0.738$	$5.023_{d} \pm 0.615$	$4.543_{d} \pm 0.762$	4.990	*
Tuber/root ratio (TRR)	$0.040_{a} \pm 0.026$	$0.637_{bd} \pm 0.073$	$0.225_{b} \pm 0.085$	$0.451_{bd} \pm 0.044$	$0.481_{bc} \pm 0.060$	$0.558_{cd} \pm 0.039$	17.189	***
Number of tubers	$0.286_{a} \pm 0.184$	$1.571_{a} \pm 0.297$	$0.858_{a} \pm 0.261$	$1.286_{a} \pm 0.184$	$2.000_{a} \pm 0.309$	$5.143_{b} \pm 1.299$	3.063	*n.s*
Tuber fresh mass (g)	$0.009_{ab} \pm 0.006$	$0.399_{ab} \pm 0.091$	$0.075_{ab} \pm 0.030$	$0.541_{ab} \pm 0.107$	$4.597_{b} \pm 1.221$	$11.866_{c} \pm 2.709$	7.630	**
Basal stem diameter (mm)	$1.129_{a} \pm 0.083$	$1.283_{a} \pm 0.063$	$1.236_{a} \pm 0.062$	$1.371_{a} \pm 0.084$	$3.660_{b} \pm 0.234$	$3.236_{b} \pm 0.285$	2.080	*n.s*
Stem length (cm)	$7.143_{a} \pm 0.969$	$16.428_{bc} \pm 3.176$	$7.329_{a} \pm 0.997$	$31.958_{ac} \pm 3.755$	$99.786_{c} \pm 35.862$	$326.500_{d} \pm 38.305$	20.430	***
Number of branches	$0.000_{a} \pm 0.000$	$0.143_{a} \pm 0.143$	$0.143_{a} \pm 0.143$	$0.429_{a} \pm 0.202$	$2.143_{b} \pm 0.340$	$3.857_{c} \pm 0.404$	7.837	**
Apical dominance index	N/A	N/A	N/A	N/A	$1.1471_{a} \pm 0.436$	$6.461_{b} \pm 3.883$	3.191	*n.s*
Leaf area (cm^2)	$6.074_{ac} \pm 1.254$	$4.100_{ac} \pm 0.954$	$7.234_{ac} \pm 0.697$	$4.571_{ac} \pm 1.356$	$39.747_{b} \pm 3.194$	$5.288_{c} \pm 0.922$	60.977	***
Leaf fresh mass (g)	$0.086_{ac} \pm 0.018$	$0.062_{ac} \pm 0.013$	$0.116_{ac} \pm 0.014$	$0.067_{ac} \pm 0.021$	$0.562_{b} \pm 0.054$	$0.076_{c} \pm 0.015$	55.677	***
Leaf dry mass (g)	$0.027_{a} \pm 0.006$	$0.019_{a} \pm 0.003$	$0.052_{ac} \pm 0.008$	$0.020_{ac} \pm 0.007$	$0.192_{b} \pm 0.029$	$0.022_{c} \pm 0.005$	39.144	***
Specific leaf area	$248.93_{a} \pm 26.260$	$231.901_{a} \pm 27.795$	$173.174_{ab} \pm 20.3$	$251.3_{a} \pm 24.819$	$224.211_{a} \pm 23.352$	$320.035_{ab} \pm 45.317$	3.180	*n.s*

Leaf matter per area	$0.004_{a} \pm 0.0003$	$0.005_{a} \pm 0.001$	$0.008_{ab} \pm 0.002$	$0.005_{a} \pm 0.0002$	$0.005_{a} \pm 0.001$	$0.004_{a} \pm 0.001$	0.434	*n.s*
Leaf water content (g)	$0.059_{ac} \pm 0.012$	$0.043_{ac} \pm 0.011$	$0.064_{ac} \pm 0.014$	$0.047_{ac} \pm 0.014$	$0.370_{b} \pm 0.030$	$0.054_{c} \pm 0.011$	52.280	***
Leaf dry matter content (mg g^{-1})	$32.871_{a} \pm 4.009$	$31.619_{a} \pm 2.083$	$51.44_{a} \pm 14.085$	$31.153_{a} \pm 1.317$	$33.377_{a} \pm 2.136$	$27.057_{a} \pm 2.368$	0.037	*n.s*
Shoot mass ratio	$0.468_{a} \pm 0.061$	$0.546_{ab} \pm 0.056$	$0.684_{ab} \pm 0.031$	$0.609_{ab} \pm 0.050$	$0.701_{b} \pm 0.027$	$0.636_{b} \pm 0.046$	1.778	*n.s*

LP appears to have allocated a significantly higher percentage of its biomass belowground at 5 months; while, SP invested significantly more biomass to tubers than LP at the same time **(Table 5.1 and Figure 5.1d).** Belowground biomass ratio (BMR) in LP gradually decreased while it increased in SP between 10 and 18 months respectively. After 5 and 10 months of growth, the proportion of tuber to root ratio (TRR) was significantly higher for SP than LP, but after 18 months TRR values were similar **(Figure 5.1c)**. There was no significant difference in the shoot mass ratio (SMR) between the two forms **(Table 5.1);** however the leaf area ratio (LAR) for LP was significantly higher than that of SP over time **(Figure 5.1e).** Specific leaf area (SLA) did not differ significantly at 5 months but differed significantly after this age, with SP having a higher SLA than LP. Leaf dry matter content (LDMC) or leaf area matter (LMA) was not significantly different between the two forms, except at 10 months when LP showed significantly higher LDMC than SP **(Figure 5.1 and Table 1)**.

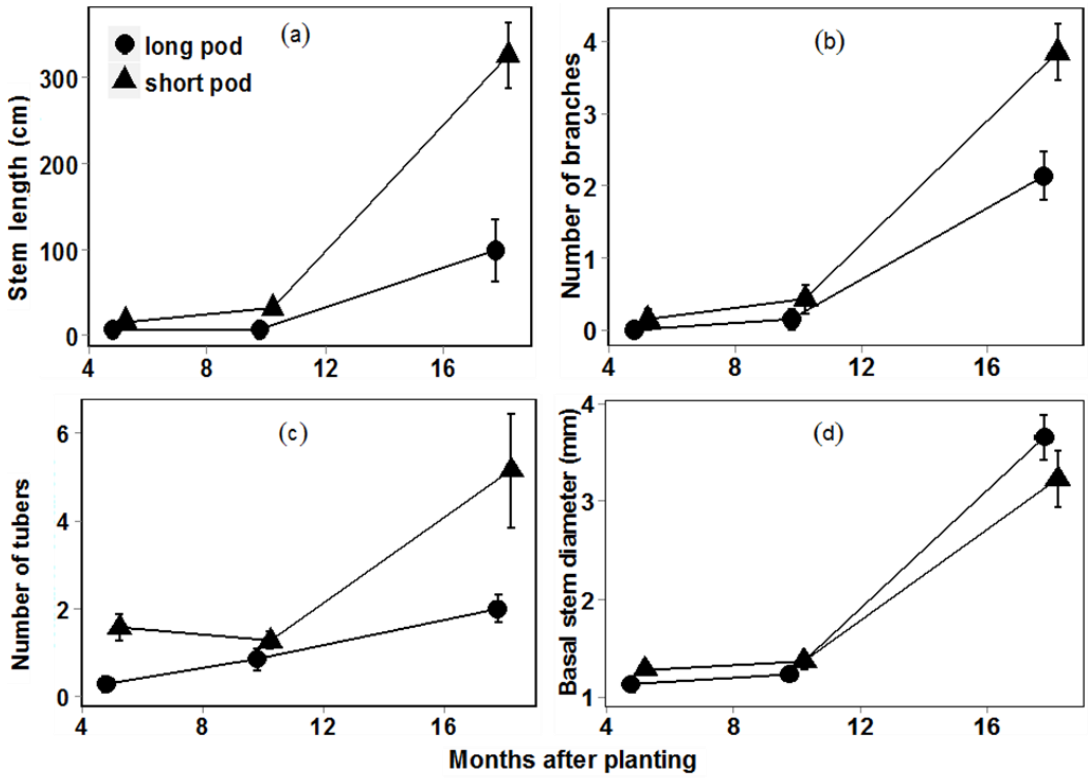


Figure 5.2. The pattern of resource allocation of LP and SP plants of varying ages in months, (mean ± SE, N=7). (a) Maximum stem length (cm); (b) Number of branches; (c) Number of tubers (d) Basal stem diameter – (BSD) (mm).

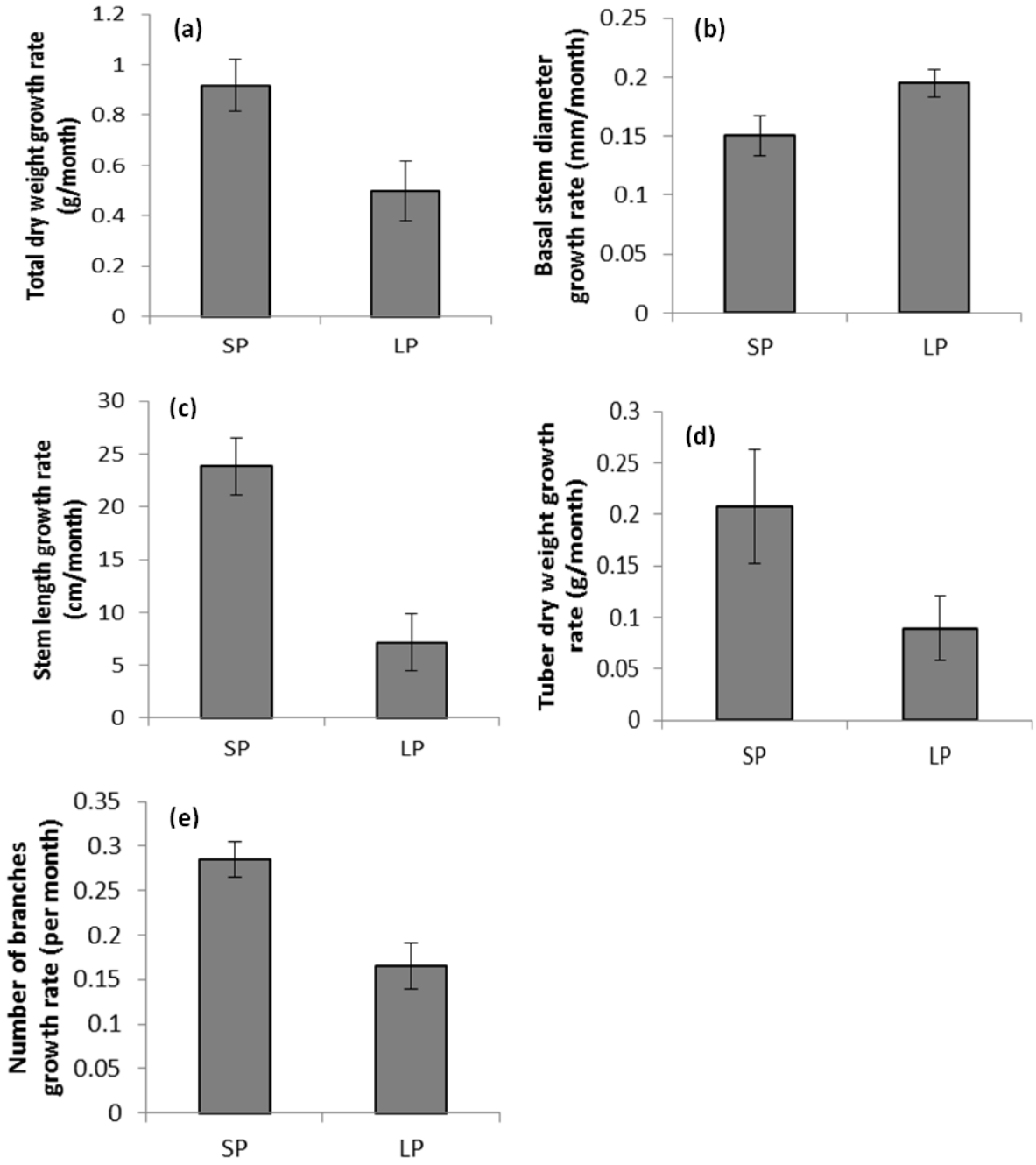


Figure 5.3. Comparison of absolute change of variables between long pod (LP) and short pod (SP) plants in the glasshouse (mean ± SE, N=7) calculated between 10 and 18 months: (a) change in total dry weight per month; (b) change in basal stem diameter (BSD) per month; (c) change in stem length per month; (d) change in tuber dry weight per month and (e) increase in the number of branches per month.

Growth parameters

Except for BSD, other growth related traits such as number and size of tubers, length of stems, and number of branches differed significantly between 10^{th} and 18^{th} month old LP and SP **(Figure 5.2a, b, c, d).** ADI, an indicator of branching architecture was significantly different only after 18 months **(Table 5.1),** but could not be calculated for 5 and 10 months due to lack of branching in LP and an insignificant number of branches for SP **(Figure 5.2b)**.

Estimates of growth rate such as change in total biomass ($F_{1, 39} = 47.03$, $p < 0.001$), stem length ($F_{1, 39} = 47.05$, $p < 0.0001$) tuber dry weight ($F_{1, 39} = 19.43$, $p < 0.005$) and number of branches ($F_{1, 39} = 61.49$, $p < 0.0001$) differed significantly between the two forms over time **(Figure 5.3a, c, d, e).** SP showed a higher rate of change in total biomass, stem length and tuber biomass than LP **(Table 5.1).** Change in BSD did not differ significantly between the two varieties over time **(Figure 5.3b).**

Overall, the observed differences between LP and SP can be summarized by the PCA graphical representation **(Figure 5.4)**, where traits of both forms largely overlap at 5 and 10 months but SP can be clearly distinguished at 18 months. PC1 (the principal axis of variation) together with PC2 explained about 60% of the total variation of the data (**see Figure 5.4 and Table 5.2).** Some of the traits that were positively associated with PC1 were total dry mass, tuber dry mass, number of branches, stem length and basal stem diameter. These traits are indicators of relative growth of a plant, in terms of mass and height. PC2 was positively correlated with apical dominance index, root mass ratio and number of tubers while negatively associated with shoot/root ratio, shoot mass ratio and basal stem diameter **(Table 5.2)**.

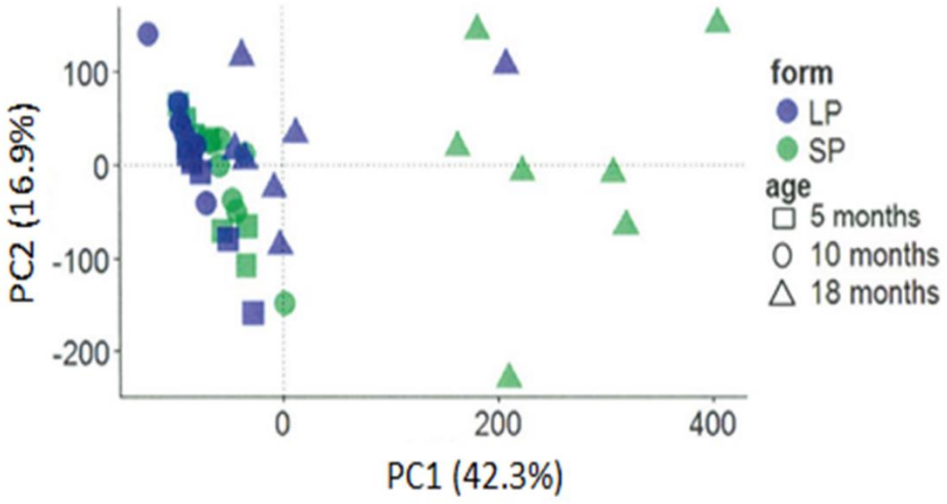


Figure 5.4. Graphical representation of the first and second PCA axes of different plant traits analysed for form (LP vs SP) and age of the plants (5, 10 and 18 months).

5.5 Discussion

The SP form, which is more widely distributed within eastern Australia, showed faster growing strategies. Higher values of RGR, stem length, number of tubers, and SLA are indicators of a successful colonizer. Higher values of RGR normally correlate with high values of leaf area ratio (LAR) and SLA (Garcia-Serrano *et al.* 2005). The results described in this Thesis are thus in accordance with the predictions of the 'leaf economic spectrum' (LES) hypothesis (Wright *et al.* 2004), which suggests a fundamental trade-off in the traits held by fast- and slow-growing plant species. According to the LES theory, where a species can be found within the spectrum is associated with strategies for resource capture and use. At one extreme, species are faster growing and highly productive, while on the other end slower growing and more conservative species occupy (Holaday *et al.* 2015).

Table 5.2. Principal component loadings of the data set, eigenvalues and their contributions to the correlations, showing only the first four components

Traits	PC1	PC2	PC3	PC4
Total dry mass (g)	1.037	0.211	0.184	-0.086
Shoot dry mass (g)	1.021	0.089	0.134	-0.061
Root dry mass (g)	0.994	0.272	0.292	-0.099
Tuber dry mass (g)	0.942	0.424	0.206	-0.124
Shoot mass ratio	0.294	-0.749	-0.492	-0.243
Root mass ratio	-0.632	0.417	0.704	0.222
Tuber mass ratio	0.465	0.334	-0.328	-0.002
Shoot/root ratio	0.409	-0.471	-0.671	-0.095
Tuber/root ratio	0.647	0.042	-0.548	0.001
Number of tubers	0.838	0.460	-0.037	-0.229
Basal stem diameter (mm)	0.927	-0.266	0.232	0.129
Stem height (cm)	0.844	0.217	-0.083	-0.114
Number of branches	0.974	0.116	0.035	-0.040
Apical dominance index	0.588	0.528	0.109	-0.245
Leaf area (cm^2)	0.517	-0.757	0.457	0.313
Leaf area ratio (cm^2g^{-1})	-0.637	-0.128	0.268	-0.173
Specific leaf area (cm^2g^{-1})	0.285	0.354	-0.402	0.569
LDMC (mg g^{-1})	-0.275	-0.232	0.422	-0.878
Importance of components				
Eigen values	11.811	4.729	3.523	2.220
Proportion explained	0.422	0.169	0.126	0.079
Cumulative proportion	0.422	0.591	0.717	0.796

Recent evidence, however, suggests the same carbon assimilation strategies are used by invasive and non-invasive plants (Leishman *et al.* 2010), but invasive plants have a tendency to cluster towards the 'high return on investment' end of the world wide leaf economic spectrum (Funk *et al.* 2013). Although SP seems to lean towards this end of the spectrum for some traits at 18 months, there were significant overlaps with LP earlier in the plants' growth. Most studies simply consider 'adult' traits (e.g. Bachmann *et al.* 2012; Burns 2006; Hulshof and Swenson 2010), so we know very little about younger plants (but see Luo *et al.* 2015). In this study, there is evidence that trait differences are minimal up to 10 months old, but after this age our results suggest that they begin to differ between LP and SP. In our study, PCA shows that the two forms are different at 18 months with the variation mostly explained by growth related traits (PC1), followed by difference in how biomass is allocated below- and above-ground (PC2).

Our results also seem to contradict findings by Taylor and Dhileepan (2012) who observed that LP had higher growth rates than SP in the field. These differences could be attributable to environmental (Evans and Hughes 1961) and growing conditions (field vs glasshouse) (Limpens *et al.* 2012). Moreover, whilst we generated experimental plants from seeds (seedlings) in our experiments, Taylor and Dhileepan (2012) used plants grown from tuberlings. Also, in the current experiment, plants were not supported while in Taylor and Dhileepan (2012) they were supported with trellises. Our study could also be limited by lack of additional nutrients in the commercial potting mix, although all individuals in the experiment were treated the same and therefore growth and response is comparable.

Although SP had slightly higher values of SLA, it had lower values of LAR when compared to LP. Because LAR is a measure of the leafiness of a plant (Radford 1967), our results imply that although LP might be leafier, SP invests more biomass to branches and stems, which could be a benefit for growing taller and spreading wider. Higher SLA has been positively correlated with high RGR and more rapid turnover of leaf material (Grotkopp *et al.* 2002). By rapid growth and quick tissue turnover, plants ensure that they outcompete others for limited resources (Gallagher *et al.* 2015). High growth rates by more successful species are particularly important in the seedling stage of a plant's life history (Grotkopp *et al.* 2002). Developing more branching is highly advantageous for vines as it is a way to increase LAR and LMR for maximum harvesting of light in order to optimise photosynthesis. Our results partly corroborate this hypothesis as we found that SP displayed higher values for SLA and LMR (but not for LAR) than LP. By developing more branches than LP (indicated by higher ADI values), SP can effectively out-compete other competitors in the environment for limiting resources.

Transformer plants such as vines like *D. unguis-cati*, thrive in growing vertically and spreading horizontally to monopolise light environments (Heckel 2004). The negative impacts of this group of plants lie in their ability to smother host tree canopies that they use as supporting structures (Harris and Gallagher 2011; Harris *et al.* 2007; Zhang *et al.* 2004). *Dolichandra unguis-cati* forms thick mats of intertwining creeping stems and branches on forest floors (Osunkoya *et al.* 2009). Thus, ensuring rapid elongation of stems and a higher branching architecture may be central to the successful colonization of empty habitats by SP. This pattern of growth reduces light availability to low lying vegetation and may prevent

recruitment of native plants (Downey and Turnbull 2007; Schnitzer and Bongers 2002; Zhang *et al.* 2004).

This study shows that SP develops subterranean tubers early in its development while LP seems to delay tuber development. Tubers are used as a sink or storage organs for moisture and photo-assimilates and they may also regenerate producing new plants (Janeček and Klimešová 2014; Orthen 2001; Schubert and Feuerle 1997). Apart from seed germination (Buru *et al.* 2014; Vivian-Smith and Panetta 2004b), *D. unguis-cati* propagates vegetatively through tubers (Downey and Turnbull 2007; Osunkoya *et al.* 2009). Horizontal stems and branches trailing along the ground develop roots at nodes, which in turn develop tubers. If the new plants regenerating at the nodal tubers are severed from the mother plant, they grow independently as genets. This study shows that SP develops significantly more tubers per plant than LP, which could be a clonal survival strategy to increase its competitiveness. Clonal growth of a species may enhance its invasion success by way of rapid formation of monocultures (Aguilera *et al.* 2010; Pyšek and Richardson 2007). Liu *et al.* (2006) found a positive relationship between clonality and invasiveness. They found that more than 66% of the most invasive plants they studied in China were clonal. Resource storage by clonal plants function as a back-up measure in case of adverse alterations in the growth conditions of the plant (Suzuki and Stuefer 1999). Tubers can also remain dormant for extended periods belowground as a stress tolerance strategy (Orthen 2001).

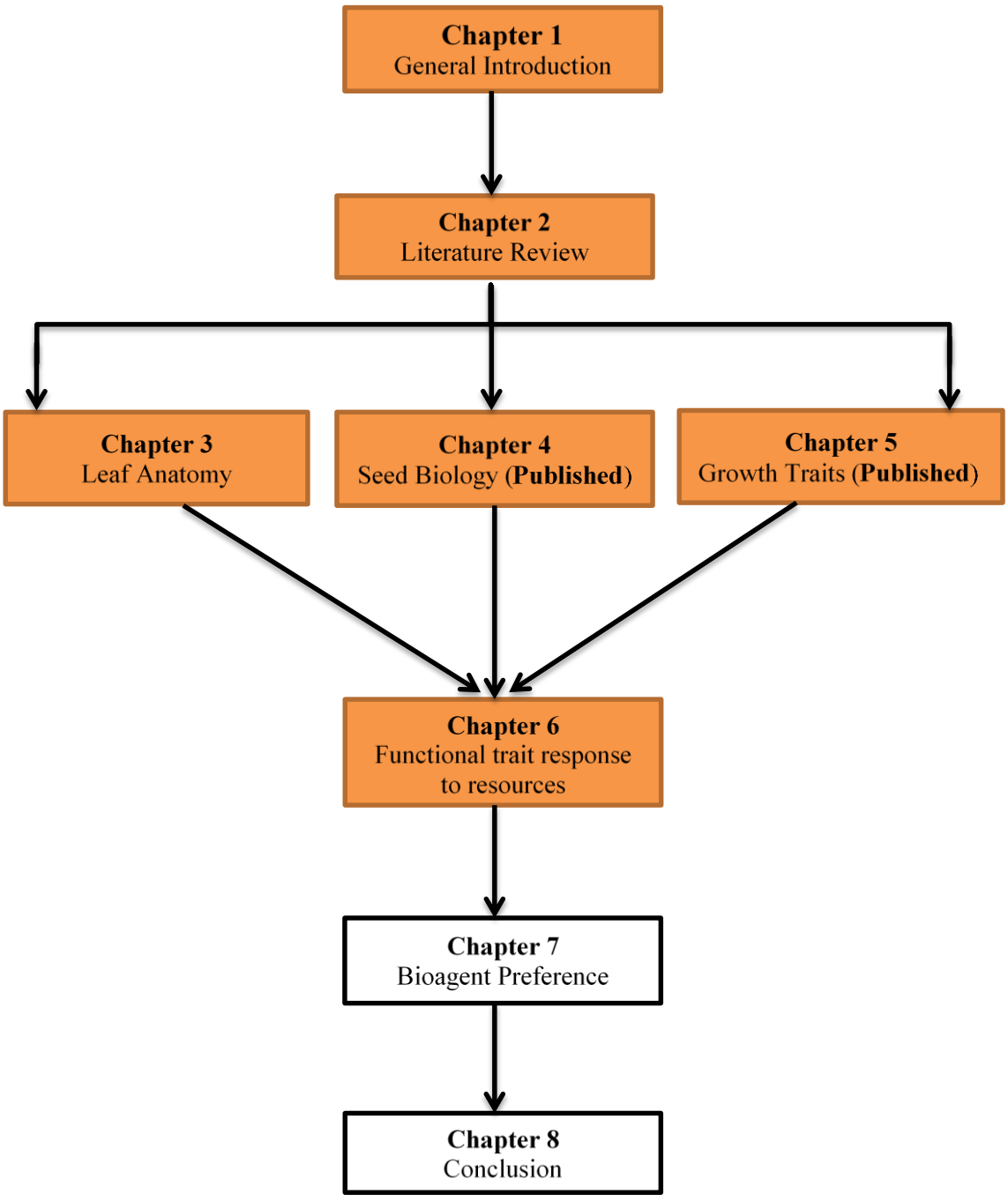
Conclusions

Previous studies have shown SP to exhibit more rapid and higher germination rates than LP at various temperatures (Buru *et al.* 2014) and a higher frequency of polyembryony than LP (Buru *et al.* 2016). Seeds of the two forms do not differ in their average mass (Shortus and Dhileepan 2011). This study has shown that SP displayed superior values of traits known to be associated with successful invaders (Chun *et al.* 2007; van Kleunen *et al.* 2015). Therefore it may be safe to assume that were the two forms to be introduced into novel environments at the same time, SP would likely be more successful in colonizing the habitats than LP (Gallagher *et al.* 2015; Godoy *et al.* 2012; Kolar and Lodge 2001; Pyšek and Richardson 2007; van Kleunen *et al.* 2010b). Thus, our results partly explain why SP seems to be abundant in

Australia, although LP is postulated to also have a potential to become widespread if not carefully managed (see Taylor and Dhileepan 2012).

Acknowledgements

We acknowledge both the Government of Botswana and the Department of Agriculture and Fisheries (Queensland, Australia) for part-sponsorship of this study through student scholarships to JC Buru. We thank members of the Plant Structure and Systematics Research Group and Dr. Tanya Scharaschkin for providing valuable feedback on earlier versions of the manuscript. We also thank three reviewers who gave very useful comments that helped improve the manuscript greatly.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Chapter 6: Physiological performance of the two forms of an invasive vine, *Dolichandra unguis-cati* in response to variable resource conditions

6.1 Abstract

It is commonly agreed that invasive species are more efficient at resource acquisition and use, which results in their faster growth than non-invasive species. In this study, two forms of *Dolichandra unguis-cati* that show different levels of invasiveness were used as case study to test this hypothesis. A trait based approach was followed and a partial factorial experiment was conducted in a glasshouse. The long pod and short pod forms were grown under two levels of light, water and nutrient resources. The experiment lasted for approximately 15 months and physiological and biomass traits were measured. Short pod exhibited higher values of carbon assimilation, water use efficiency and leaf nitrogen than long pod. However, long pod produced more biomass than short pod, with the difference driven strongly by high resource conditions. Phenotypic integration did not differ between long pod and short pod in general, but short pod exhibited significantly higher phenotypic integration when high light and nutrients were considered alone. Short pod developed a significantly higher number of tubers than long pod. Overall short pod performed better than long pod in response to different resource conditions. This study indicates that short pod possesses traits that are well suited for a successful colonizer while long pod possess traits of opportunistic plants. This study implies that short pod and long pod use different strategies to exploit resources in the environment, therefore different carbon economies. The results presented in this chapter contribute to our understanding of the prevalence of short pod in Australia.

6.2 Introduction

The short pod (SP) form of *D. unguis-cati* is the most prevalent in Australia, occurring extensively in Queensland and New South Wales (Dhileepan 2012) while the long pod (LP)

form occurs in ~15 isolated sites in southeast Queensland (Dhileepan 2012; Liz Snow, personal communication, 07/03/2016). The cause for this difference in the level of prevalence of these forms is unknown. However, given its higher prevalence, we predict that SP will be better at utilising resources (e.g. pulses in light gap and nutrient load) in the environment resulting in faster growth than LP. We expect SP to show high resource use efficiency and have a faster growth regime in response to light, water and nutrient pulses.

In this study, we set out to determine the physiological and biomass responses of LP and SP to varying levels of light, water and nutrient resources. We used two partially factorial experiments to compare physiological performance such as carbon assimilation rates, resource use efficiency traits, biomass allocation patterns and trait correlations between the two forms. LP and SP present a perfect system to test whether the more abundant form would exhibit higher values of invasiveness traits and be better at utilisation of resources than the uncommon form (Muth and Pigliucci 2006; van Kleunen *et al.* 2010a). This is more so because these forms have overlapping life history traits and co-exist in Australia (Shortus and Dhileepan 2011).

One of the widely accepted hypotheses that explain invasiveness of species is that such species are better at resource acquisition and use in response to environmental changes (Durand and Goldstein 2001; Funk and Vitousek 2007; Osunkoya *et al.* 2010b). Adaptive trait plasticity in response to a suite of environmental conditions has also been closely associated with colonization success (Chun *et al.* 2007; Molina-Montenegro *et al.* 2012; Sultan and Matesanz 2015). Phenotypic plasticity refers to the ability of a single genotype to modify its phenotypes in response to prevailing abiotic factors in the environment (Abakumova *et al.* 2016; Bradshaw 1965; Nicotra *et al.* 2010). High trait plasticity is positively correlated with colonization success as it encourages rapid spread into environmentally heterogeneous habitats (Godoy *et al.* 2011). Richards *et al.* (2006) classify invasive species into three categories that may describe how an invasive plant may benefit from trait plasticity. These are 1) Jack of all trades: Species that maintain fitness in adverse environments, 2) Master of some: Species under this category can increase their fitness under favourable conditions only, and finally, 3) Jack and Master: Species in this category have some characteristics of 1 and 2.

Whereas there is a general consensus that trait differences exist between invasive species and non-invasive ones (Drenovsky *et al.* 2008; Hulshof and Swenson 2010), this conclusion does not always hold (Meiners 2007; Palacio-López and Gianoli 2011). Others have found that traits are only different between invasive and non-invasive species under certain environmental

conditions (Melinda and Alan 2001) and that both invasive and non-invasive plants have similar strategies of carbon capture (Leishman *et al.* 2010). A universal leaf economic spectrum (LES) of carbon gain (i.e., assimilation and plant growth) has been demonstrated, ranging from species with low to fast growing strategies (Westoby and Wright 2006; Wright *et al.* 2004). According to the LES theory, the position a species occupies on this spectrum is associated with strategies for resource capture and use. On one end of the spectrum are the highly productive, fasting growing plants (Reich 2014), while more conservative and slow growing species occupy the other end of the spectrum (Holaday *et al.* 2015). Invasive species have been shown to occupy the 'high return on investment' end of the LES with high SLA, RGR, trait plasticity and carbon gain (Funk *et al.* 2013; Leishman *et al.* 2007; Penuelas *et al.* 2010).

Studies have found that disturbed habitats were more prone to invasion by non-native invasive species (Hamilton *et al.* 2005; Lake and Leishman 2004). These disturbances could manifest in the form of nutrient enrichment (Hobbs and Huenneke 1992) or alterations in moisture and light levels (Gatti *et al.* 2015; Kettenring *et al.* 2015; Masaki *et al.* 2015). The correlation between high nutrient availability and colonization success (Burns 2004; Liu *et al.* 2015) is predicated on the hypothesis that native species have local adaptation to low nutrient status. Consequently, these species are unable to compete efficiently under high nutrient scenarios (Alpert *et al.* 2000; Davidson *et al.* 2011). This is in agreement with the theory of fluctuating resource availability proposed by Davis *et al.* (2000), which suggests that where there are pulses of unused resources, habitats become susceptible to invasion by non-native species (also see Elst *et al.* 2016). Under this scenario, invasive species are better competitors than non-invasive ones (Vila and Weiner 2004), and can acquire and efficiently use extra resources to enhance their performance and reproduction (Dawson *et al.* 2015a).

Lake and Leishman (2004) suggested that disturbed habitats with excess nutrient resources are likely to be colonised by species with the capacity for fast growth such as vines. As vines are not self-supportive, they depend on other plants and physical structures to reach certain heights (Harris and Gallagher 2011; Harris *et al.* 2007). Thus, where there are additional nutrients, vines and climbers maximise growth (Lake and Leishman 2004), often investing more on aboveground structures, especially their stems, branches and leaves (Campanello *et al.* 2016; Ichihashi and Tateno 2015; Schnitzer 2015). Under low light conditions, vines have been shown to adopt a "searching" growth strategy where they reduce allocation to branches and leaves but increase stem length (Baars and Kelly 1996). Under high light, they adopt an

"exploiting" strategy by allocating more resources to leaves and branches to harvest more light resources (Lee 1988). The negative impacts of invasive vines result from their capacity to smother their host plant canopies (Zhang *et al.* 2004), at times also creating thick mats of intertwining creeping stems on forest floors (Campbell *et al.* 2015; Downey and Turnbull 2007; Schnitzer and Bongers 2002).

It is commonly agreed that invasive species have a range of life history performance and fitness traits that enable them to colonize novel habitats (Funk and Zachary 2010). These traits include high photosynthetic rates (or carbon assimilation), relative growth rates (RGR), specific leaf area (SLA) (Osunkoya *et al.* 2014; van Kleunen *et al.* 2010a) and high resource use efficiency (Firn *et al.* 2012; Funk and Vitousek 2007).

We addressed the following specific questions in this study:

1. How do LP and SP compare in biomass production and allocation patterns, e.g. total dry mass, shoot/root ratio under different light, water and nutrient treatments?
2. What is the level of variation in leaf functional traits and coordination of these traits under different resource conditions for LP and SP?
3. Is there any difference in carbon assimilation and chlorophyll fluorescence traits between LP and SP under variations of light, water and nutrient levels?
4. Do LP and LP show different physiological trait responses to short-term pulses of light?

We expected that SP, the most abundant form of *D. unguis-cati* would exhibit higher physiological and performance traits in response to light, water and nutrient resources than LP.

6.3 Materials and Methods

This study was conducted at the shade and glasshouse facility of Department of Agriculture, Forestry and Fisheries (DAFF), Ecosciences Precinct (GPS coordinates: 27°29'41.5248'' S; 153°1'49.2132" E) in Brisbane, Australia. An overview of the weather conditions of Brisbane City has been described in Chapter 5 of this thesis.

Experimental design

Seeds of LP and SP were collected from various sites around the greater Brisbane area in southeast Queensland, Australia. Once collected, seeds were stored for two weeks at room temperature in paper envelopes that were placed in containers with silica gel to ensure they were dry before germination commenced. Voucher specimens of plant samples collected from each site have been lodged with the Queensland Herbarium (BRI). Voucher specimens obtained from Oxley and Carindale localities correspond to those lodged by Boyne *et al.* (2013a).

Seed germination conditions were discussed in detail in Chapter 4 of this thesis. After two weeks of germination, seedlings were transferred into small 0.8 L plastic pots (dimensions: Diameter = 200mm, Height = 190mm) filled with locally available commercial seed raising potting mix (Osmocote©) for two months to establish the plants. Plants were regularly watered every two days with no additional nutrients added at this point.

After two months of growth, plants of both forms were transferred into bigger 13.5 L plastic pots (dimensions: Diameter = 300 mm, Height = 290 mm) filled with a multi-purpose potting mix containing a wetting agent and trace elements (Osmocote©). These plants were then subjected to different treatments as described below.

Light x Nutrients Experiments

This experiment was set up in a shade house at the Ecosciences Precinct Facilities. The shade house does not have temperature or humidity control, rather, it experiences the prevailing conditions. Average temperatures during the warmer months (October – April) ranged from 18 °C to 35 °C and between 10 °C and 23 °C during the cooler months (May – September). Relative humidity ranged between 50 – 70% during this study.

Two light levels were used in this experiment, i.e. (a) High light (HL): Plants received ~35-40% of full sun (870 -1100 µmol m^{-2} s^{-1}); (b) Low light (LL): Plants received ~1-2% of full sun (25-50 µmol m^{-2} s^{-1}). LL conditions were made by creating a shade using 2-3 layers of 90% shade cloth. The amount of light was measured using a LICOR 6400 portable photosynthesis system (LICOR, Inc., Lincoln, NE).

For both the high and low light conditions, two nutrient levels of treatment were applied. High nutrients (HN): Potting mix enriched with nutrients by adding granules of a slow-release all purpose fertiliser (Osmocote, NPK 21:2:6 plus trace elements) every two weeks. Low

nutrients (LN): No additional nutrients were added to the growth media. For both light treatments, plants were maintained at 100% pot capacity by receiving ~ 300ml of water every two days using an automated/programmed watering system. The combinations of treatments were as follows: HLHN, HLLN, LLHN and LLLN.

Water x Nutrients Experiments

These sets of experiments were performed in a temperature controlled glasshouse facility at Ecosciences Precinct. During the experiment in the glasshouse, the temperature ranged from 22-28 °C. The mid-daytime photosynthetically active radiation (PAR) in the glasshouse ranged from 800 – 1500 µmol m^{-2} s^{-1}. These light conditions are equivalent to the high light conditions under HLHN described above. Two different water regimes were applied to both LP and SP. Well-watered or high water (HW): Plants were maintained at 100% pot capacity by receiving ~ 300 ml of water every two days. Low watering (LW): Plants were maintained at 5 % pot capacity by receiving ~ 15 ml of water once every two weeks.

Pot water capacity was determined at the beginning of the experiment by filling four replicate 13.5 L plastic pots with the commercial potting mix (Osmocote Multi-Purpose). The soil was oven dried using a Thermolite Scientific + 6100 Model oven for 48 hours at 80 °C and weighed to determine dry weight (DW). The potting mix was saturated with water and excess water allowed to drain freely for 2-3 hours until no more water drained. The pots were weighed again to determine saturated weight (SW). Pot capacity was calculated as the difference between SW and DW (Frosi *et al.* 2013). For each level of water treatment, two levels of nutrients treatment were applied as described for the light experiments above. The combinations of treatments were as follows: HWHN, HWLN, LWHN and LWLN. Both the light*nutrients and water*nutrients treatments were replicated seven (7) times for both LP and SP. Experiments commenced between October and November 2014 and were terminated in January 2016, (~14 months after the initiation of the experiment). Growth traits, leaf physiological and chemistry data were collected at the end of the experiment.

Physiological traits

Leaf gas exchange coupled with chlorophyll fluorescence measurements were taken using an open portable photosynthesis gas exchange system (LI-6400; LICOR, Inc., Lincoln, NE) connected to a fluorescence chamber head (LI-6400-40 leaf chamber fluorometer; LI-

COR, Inc.). For each treatment, five replicates of each form (LP vs. SP) were randomly chosen, and for each replicate plant, two recently matured leaflets were identified and tagged for measurements. Photosynthetic rate (A_{max}, µmol m^{-2} s^{-1}), transpiration rate (E, mol m^{-2} s^{-1}), non-photochemical quenching (NPQ = F^{o}_{m}-F'_{m}/F'_{m}), stomatal conductance (g_s, mol m^{-2} s^{-1}) and chlorophyll fluorescence were measured at a constant CO_2 concentration of 400 µL L^{-1} . The relative humidity within the leaf cuvette ranged between 50-65% while the temperature was kept at 23-25 °C. To investigate the response of the leaflets to changes in PAR, physiological measurements described above were taken at 50, 500, 1500 and 2500 µmol photon m^{-2} s^{-1}. From the primary physiological data collected, the following parameters were derived:

Effective quantum yield of Photosystem II (ϕPSII) calculated as:

$$\phi PSII = (Fm' - Fs')/Fm' \quad (1)$$

where Fm' is maximum fluorescence during a saturating light flash; Fs' is fluorescence yield of a light adapted leaf.

The instantaneous water-use efficiency (WUE) was calculated as follows

$$WUE = A_{max}/E, \quad (2)$$

where A_{max} is maximum carbon assimilation rate; E is transpiration rate.

Leaf chlorophyll content (measured in SPAD units) was estimated using a chlorophyll meter (Konica-Minolta SPAD-502, Spectrum Technologies, IL, USA). The same leaflets tagged for physiological measurements were used to determine chlorophyll content, taking three random measurements from each leaflet at different locations on the leaf. Physiological data could not be obtained for treatments receiving HWLN and LWLN because the plants in these treatments did not develop sufficient leaves.

After collection of physiological and chlorophyll measurements, the tagged leaflets were collected, weighed (fresh weight) and photographed against a graduated background using an IPAD camera (Apple Inc., CA, USA) for leaf area estimation. The open access software, Image J 1.47v (www.imagej.nih.gov/ij) was used to calculate the leaf area (cm^2) from images. The harvested leaflets were dried at 65 °C for 72 hours, and their dry weight measured to determine specific leaf area (SLA) and leaf dry matter content (LDMC), which were calculated following Pérez-Harguindeguy *et al.* (2013) as:

Specific leaf area (SLA) = Leaflet surface area (cm^2) / Leaflet oven dry mass (g) (3)

Leaf dry matter content (LDMC) = Leaflet dry mass (mg) / Leaflet fresh mass (g) (4)

The dried leaf samples were analysed for total carbon (C) and nitrogen (N) concentrations using Plant CN Dumas combustion method (Jung *et al.* 2003). All chemical components of this study were analysed at the Chemistry Centre, Department of Science Information, Technology and Innovation (DSITI), Ecosciences Precinct, Brisbane, Australia. After chemical analyses of the leaves and collection of the elemental data, the following parameters were calculated:

Photosynthetic nitrogen use efficiency (PNUE) = A_{max}/leaf N (5)

Leaf C to leaf N ratio (C:N) = Leaf C / leaf N (6)

Chl-to-N ratio (Chl:N) = Leaf Chl / leaf N (7)

Growth and biomass traits

Basal stem diameter (BSD) at the root-stem junction was measured in all treatments using a set of digital vernier calipers (150, 0.1 mm precision, Kincrome©). All plants were harvested and separated into aboveground (shoots) and belowground (roots + tubers) tissues. The number of tubers per plant was recorded per treatment and form. Roots and tubers were carefully washed to remove as much soil as possible, while avoiding loss of fine roots. All plant tissues were dried in an oven at 65 °C for three weeks before weighing to determine total dry mass (g) and shoot/root ratio. Total dry biomass and resultant biomass allocation parameters were calculated according to Luo *et al.* (2015) as follows:

Total dry mass (g) = Belowground dry mass (g) + shoot dry mass (g) (8)

Shoot/root ratio = Shoot dry mass (g) / (Belowground dry mass, g) (9)

Statistical Analyses

All data were tested for normal distribution and homoscedasticity using the Shapiro-Wilks test. Data that violated the ANOVA assumptions of normality and homogeneity of variance were either $\log_{10}$ transformed (A_{max}, ϕPSII, WUE), square-root transformed (stomatal conductance, shoot/root ratio) or Box-Cox power transformed (BSD, number of tubers, root and shoot dry mass, total dry mass, and all leaf traits). Values presented are back-transformed data.

Mean differences for all traits were analysed using a two-way analysis of variance (ANOVA + an error structure of replicate/leaf number/or treatment) with treatment (HLHN, HLLN, LLHN, LLLN, HWHN, HWLN, LWHN, LWLN) and form (LP or SP) as fixed effects. When significant differences were detected, a Tukey LSD post-hoc test was performed to check differences between specific means. Pearson correlation coefficients were generated to determine the linear association among important performance traits and how they compare between the LP and SP. A multivariate method of principal components analysis (PCA) was used to explore how the two forms were separated on an ordination space. A two-way ANOVA was performed on the loadings of the major axes (i.e., the first two PCA axes) with form and treatment as fixed factors. To test trait integration level for each form, Pearson correlation coefficients were generated for all physiological and growth related traits for both forms. All statistical tests were conducted using R version 3.1.0 (R Development Core Team 2014) and graphics were created using SPSS (version 22.0; IBM SPSS Statistics; Armonk, NY, USA).

6.4 Results

Biomass production and allocation patterns

Overall and across all experiments, total biomass differed significantly between long pod (LP) and short pod (LP) at the end of the growth period ($F_{1,\ 7}$ = 8.124, P<0.006; **Table 6.1**), with LP having a higher biomass accumulation, mostly driven by high light and enhanced nutrients (HLHN). Treatment had a significant effect ($F_{1,\ 88}$ = 12.195, P<0.0001) on the total biomass production of the two forms, and was of the order: HLHN>HWHN>HLLN>LWHN>LLHN>LLLN=LWLN). There was a significant interaction between form and treatment ($F_{1,\ 88}$ = 3.184, P<0.005), suggesting that response to treatments was not consistent within forms. Both forms responded better to light x nutrients than to water x nutrients treatments, as indicated by greater biomass accumulation **(Figure 6.1a, b).**

Stem diameter was significantly higher in LP than SP ($F_{1,\ 7}$ = 9.814, P<0.003), mostly driven by high light and high nutrients (HLHN). Stem diameter response to low light and nutrient resource conditions (i.e. LLLN, LWLN) was similar in both forms (**Figure 6.1c**). Stem diameter changes in response to water treatments were similar in LP and SP **(Figure 6.1d)**. Both forms responded to high resource conditions by developing a significantly higher number

of tubers **(Figure 6.1e, f)**. However, overall, SP developed more subterranean tubers than LP ($F_{1, 7}$ = 46.459, P<0.001). The Tukey LSD post hoc analysis indicate that there were no significant differences in total biomass and tuber development under the most stressful conditions of low light, low water and low nutrients **(Figure 6.1a, b, e, f)**. SP was better able to exploit extra nutrients by developing more tubers (storage organs) than LP in high light x high nutrient (HLHN) conditions **(Figure 6.1e).** This pattern was repeated in the water x nutrients conditions **(Figure 6.1f)** for SP. The LP did not show significant differences in tuber development in response to water and nutrient resources.

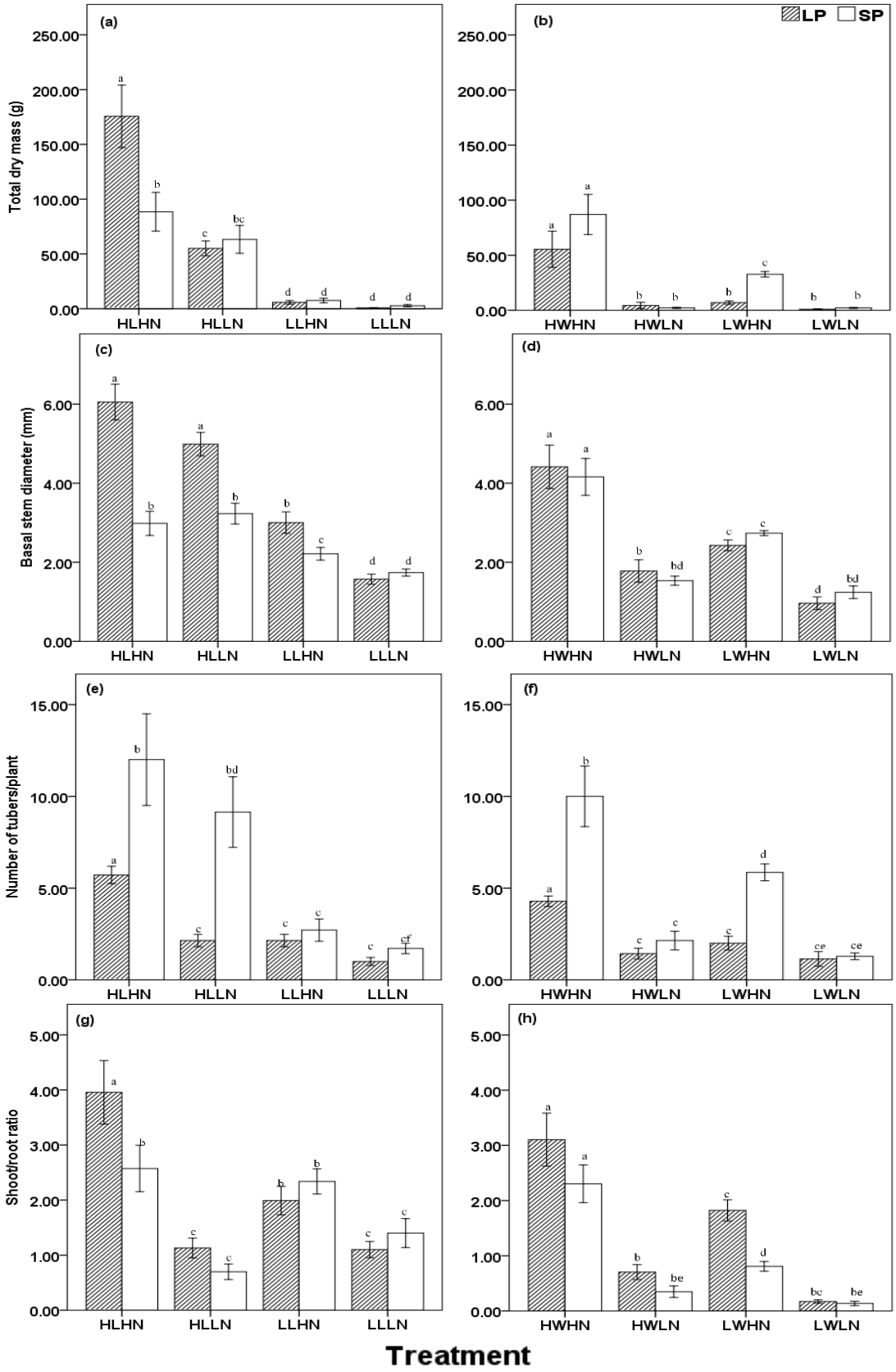


Figure 6.1. Performance traits response of LP and SP varying levels of light, water and nutrient conditions. (a, b). Total biomass accumulated; (c, d). Basal stem diameter (BSD); (e, f): Average number of tubers per plant; (g, h): Shoot/root ratio. The legend in the graph (a) applies to all graphs. Graphs on the left show traits responses to light x nutrient experiments and those on the left show trait responses to light x nutrients experiments. Similar letters above the bars indicate insignificant differences.

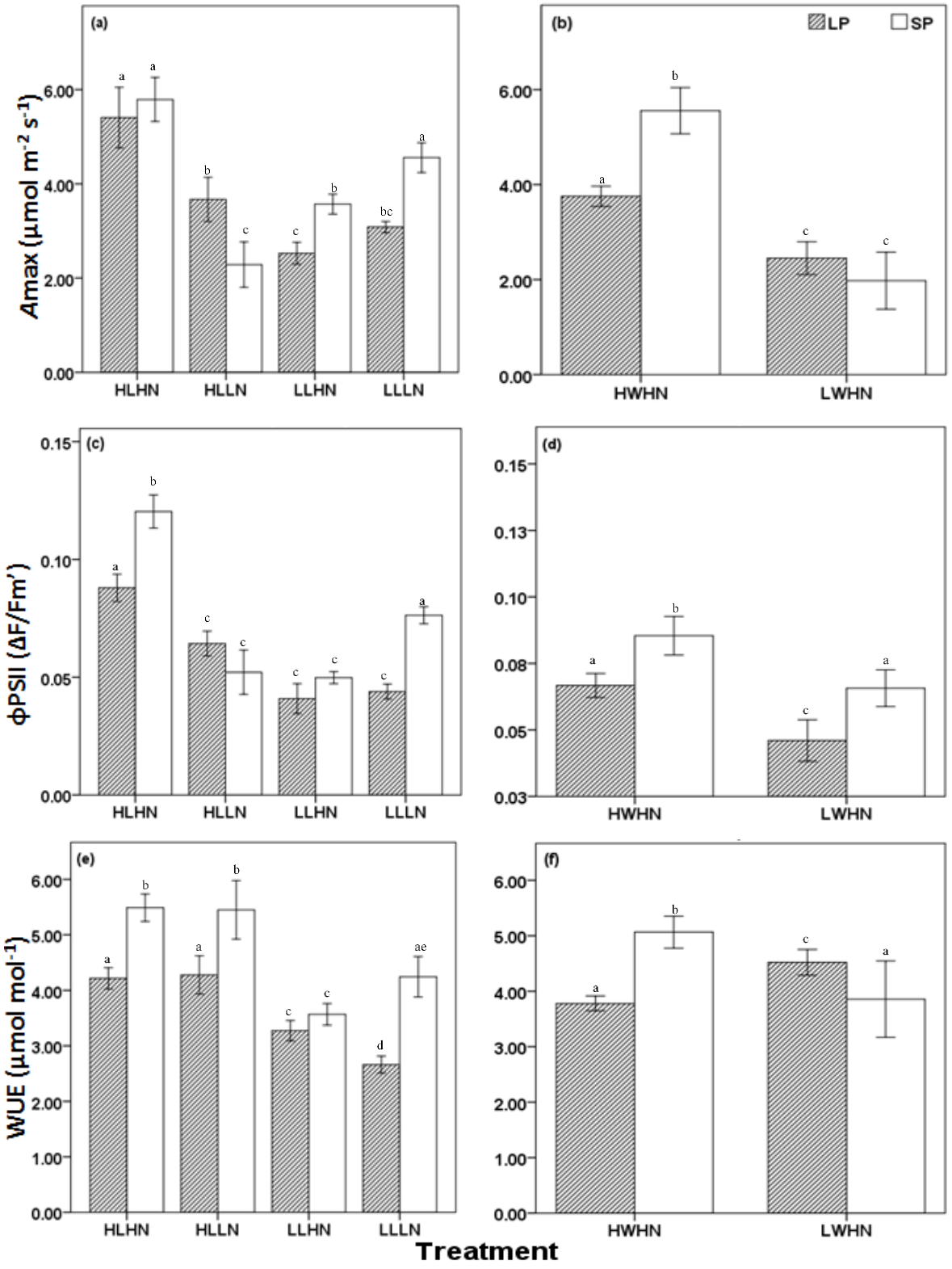


Figure 6.2. Physiological traits of LP and SP in response to differing light, water and nutrient resources. (a, b): Maximum carbon assimilation (Amax, area based); (c, d). The effective quantum yield of photosystem II, ϕ_{PSII}; (e, f): Water use efficiency, WUE. The legend in the graph (b) applies to all graphs. Graphs on the left show traits responses to light x nutrient experiments and those on the left show trait responses to light x nutrients experiments. Similar letters above the bars indicate insignificant differences.

Table 6.1 Mean (± SE) growth, physiological and leaf chemical concentrations of LP and SP. Summary ANOVA refers to F- and P-values of a two –way ANOVA with an error structure of different performance traits (BoxCox transformed), physiological traits (log_{10} transformed) for both forms of *D. unguis-cati*, with a fixed effects structure of form (LP and SP) and treatments (HLHN, HLLN, LLHN, LLLN, HWHN, LWHN)

Traits	**Form**		**Summary ANOVA**		**Direction of difference**
	LP	SP	*F*-value	*P*-value	
Total biomass (g)	38.108 ± 0.252	35.754 ± 5.884	8.124	0.006	LP>SP
Leaf area (cm^2)	26.268 ± 2.141	11.740 ± 0.799	54.519	0.0001	LP>SP
SLA ($cm^2\ g^{-1}$)	3.952 ± 0.185	4.081 ± 0.267	0.454	0.60	NS
LDMC ($mg\ g^{-1}$)	296.94 ± 14.11	293.04 ± 9.88	0.156	0.694	NS
No. of tubers/plant	2.480 ± 0.239	5.610 ± 0.688	46.459	0.0001	LP<SP
Basal stem diameter (mm)	3.149 ± 8.569	2.479 ± 0.147	9.814	0.003	LP>SP
Root dry mass (g)	11.345 ± 2.127	15.226 ± 2.535	2.536	0.122	NS
Shoot dry mass (g)	26.763 ± 6.759	20.902 ± 3.899	0.013	0.912	NS
Shoot/root ratio	1.776 ± 0.189	1.308 ± 0.145	1.988	0.20	NS
A_{max} ($\mu mol\ m^{-2}\ s^{-1}$)	3.482 ± 0.266	3.971 ± 0.342	4.067	0.05	LP<SP
A_{mass} ($\mu mol\ g^{-1}\ s^{-1}$)	15.064 ± 1.283	18.256 ± 1.849	2.960	0.09	NS
WUE ($\mu mol\ CO_2 mol^{-1}\ H_2O$)	4.029 ± 0.214	4.526 ± 0.312	30.294	0.001	LP<SP
PNUE ($\mu mol\ mol\ s^{-1}$)	1.269 ± 0.146	1.209 ± 0.075	0.138	0.71	NS
ϕPSII (*ΔF*/Fm')	0.070 ± 0.005	0.085 ± 0.007	18.20	0.0001	LP<SP
NPQ	1.711 ± 0.068	1.561 ± 0.056	24.759	0.001	LP>SP
C ($g\ m^{-2}$)	43.820 ± 0.257	43.280 ± 0.239	6.282	0.018	LP>SP
N ($g\ m^{-2}$)	3.450 ± 0.258	3.730 ± 0.185	5.310	0.03	LP<SP
C:N	14.626 ± 1.379	12.374 ± 0.821	7.289	0.01	LP>SP
N_{mass} ($mg\ g^{-1}$)	14.124 ± 1.309	15.515 ± 1.378	1.162	0.291	NS
Chl (SPAD units)	40.993 ± 0.844	57.239 ± 0.026	58.521	0.0001	LP<SP
Chl:N	13.290 ± 0.375	15.737 ± 0.769	1.599	0.215	NS

How do leaves respond to light, water and nutrient availability?

As expected, LP had a significantly higher leaf area (LA) than SP ($F_{1, 4}$ = 54.519, P<0.0001). However, specific leaf area (SLA) was similar between the two forms ($F_{1, 4}$ = 0.454, P>0.60), indicating that SP allocated more biomass per leaf area than LP. Variations in SLA were only explained by treatment ($F_{1, 4}$ = 10.879**,** *P*<0.0001), and its interaction with form ($F_{1, 4}$ = 257.845, *P*<0.0001), indicating plasticity of this trait. Both forms invested significantly less biomass in their leaves in the low light x high nutrient (LLHN) conditions when compared with

high light x high nutrients (HLHN) conditions. SLA in LLHN was two times higher than in HLHN in both forms.

As with total biomass, LP accumulated significantly higher total leaf carbon (C) than SP ($F_{1, 4} = 6.282$, $P<0.018$). Conversely, SP showed higher area based total leaf nitrogen (N) ($F_{1, 4} = 5.310$, $P<0.03$). Thus, LP exhibited significantly higher C: N ratio ($F_{1, 4} = 7.289$, $P<0.02$) than SP **(Table 6.1).** However, variations in leaf nutrient concentrations were best explained by treatment and form x treatment interactions for all leaf nutrients**.** Expectedly, total leaf N concentrations were significantly higher in HLHN than HLLN treatments, but there was no significant difference in leaf N between LLHN and LLLN for both forms.

Carbon assimilation rate and resource use efficiency in response to variations in light, water and nutrients

Despite a greater biomass accumulation by LP than SP, SP showed greater values for all physiological traits except C, C:N and PNUE **(Table 6.1)**. SP showed a greater rate of CO_2 fixation (A_{max}) on the whole ($F_{1, 5} = 4.067$, $P<0.05$). Across all treatments, variation in area based maximum carbon assimilation (A_{max}) was better explained by treatment ($F_{1, 106} = 18.554$, $P<0.0001$) and then by form x treatment interactions ($F_{1, 106} = 4.499$, $P<0.001$). Mass-based carbon assimilation rate (A_{mass}) was only marginally higher in SP ($F_{1, 4} = 2.960$, $P<0.09$). Under the light x nutrients treatments, A_{max} did not vary significantly between LP and SP, although SP showed slightly higher values in most treatments (e.g. HLHN, LLHN and LLLN) **(Figure 6.2a)**. SP had a significant shift in carbon assimilation in response to light levels in that there was a twofold difference in A_{max} between LLHN and HLHN ($P<0.0001$). Meanwhile for LP, A_{max} only increased by a factor of 0.5 from LLHN to HLHN ($P<0.001$). Under low nutrients, A_{max} increased two-fold in response to a reduction in light level only for SP ($P<0.0001$) while there was no significant change in carbon assimilation from HLLN to LLLN in LP **(Figure 6.2a)**. This trend suggests a better acclimation for SP to a decreasing light condition. A_{max} was measured for only two treatments under the water experiments, i.e. HWHN and LWHN, and the response did not vary significantly between LP and SP under these treatments **(Figure 6.2b)**. However, there was significant reduction in carbon assimilation for SP (by a factor of 3) from HWHN to LWHN ($P<0.0001$) while there was no difference between the two treatments in LP **(Figure 6.2b)**. Effective quantum yield of photosystem II (ϕ_{PSII}) was significantly higher in SP than LP (**Figure 6.2c, d; Table 6.1**).

Water use efficiency (WUE) was significantly higher in SP than LP across all treatments ($F_{1, 5}$ = 30.294, P<0.001). Variation in WUE was also significantly influenced by treatment ($F_{1, 108}$ = 11.785, P<0.001) and its interactions with form **(Table 6.1)**. Water loss in SP was more restrained under HLHN conditions, which resulted in significantly higher WUE at this treatment than at LLHN (P<0.01; **Figure 6.2e**). There was no difference in WUE between HLHN and LLHN in LP (P>030). On the contrary, in the low nutrient scenarios, WUE varied significantly between HLLN and LLLN for LP (P<0.001). SP did not show this response pattern between the same treatments **(Figure 6.2e)**. There were no differences in response to resources in the water x nutrients experiments by both forms **(Figure 6.2f)**. There was no difference between LP and SP in terms of photosynthetic nitrogen use efficiency (PNUE) ($F_{4, 31}$ = 0.138, P<0.712). Both forms exhibited lowest assimilation rates and resource use efficiencies in response to the stressful treatments involving low light and low water (LLHN, LLLN and LWHN; **Figure 6.2**).

How do physiological traits change in response to increases in PAR?

Physiological traits of both forms showed a curvilinear relationship with changes in levels of photosynthetically active radiation (PAR) **(Figure 6.3 and 6.4)**. Variation in A_{max} in response to PAR was significantly influenced by light levels ($F_{1, 455}$ = 163.0, P<0.001). The effect size of form on differences in A_{max} response was smaller ($F_{2, 455}$ = 4.63, P<0.01). A three-way MANOVA including fixed effects of form (F), treatment (T) and PAR (P) and their interactions found significant interactions between F*T ($F_{5, 108}$ = 5.49, P<0.001), F*P ($F_{1, 455}$ = 29.24, P<0.001) and F*T*P ($F_{5, 455}$ = 17.93, P<0.001), suggesting that treatment effects were not consistent across forms. This pattern signifies that forms differed in their response to treatments and depending on PAR levels.

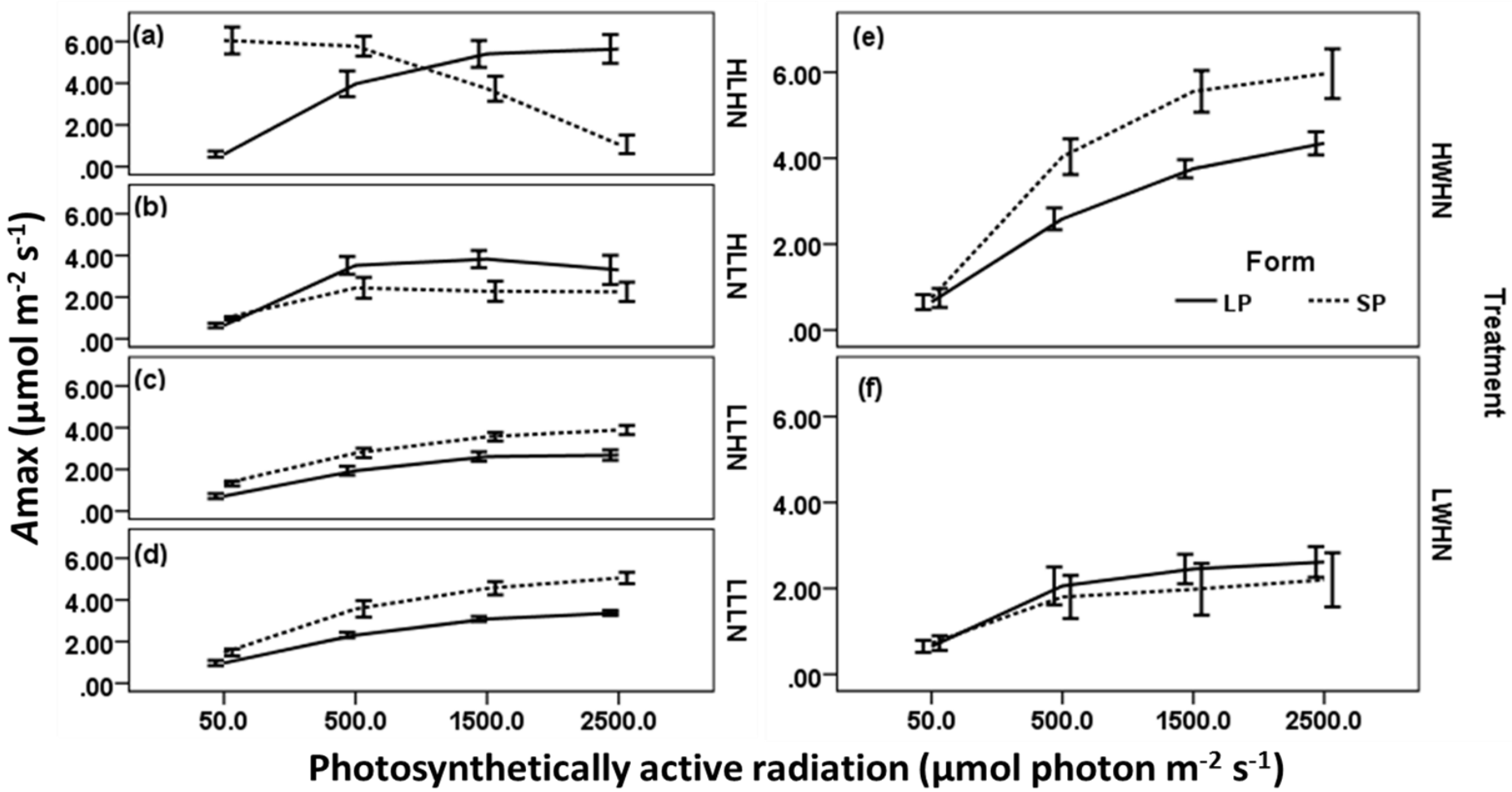


Figure 6.3. *Amax* responses of the two forms of *D. unguis-cati* growing in different light, water and nutrient resources to increasing PAR.

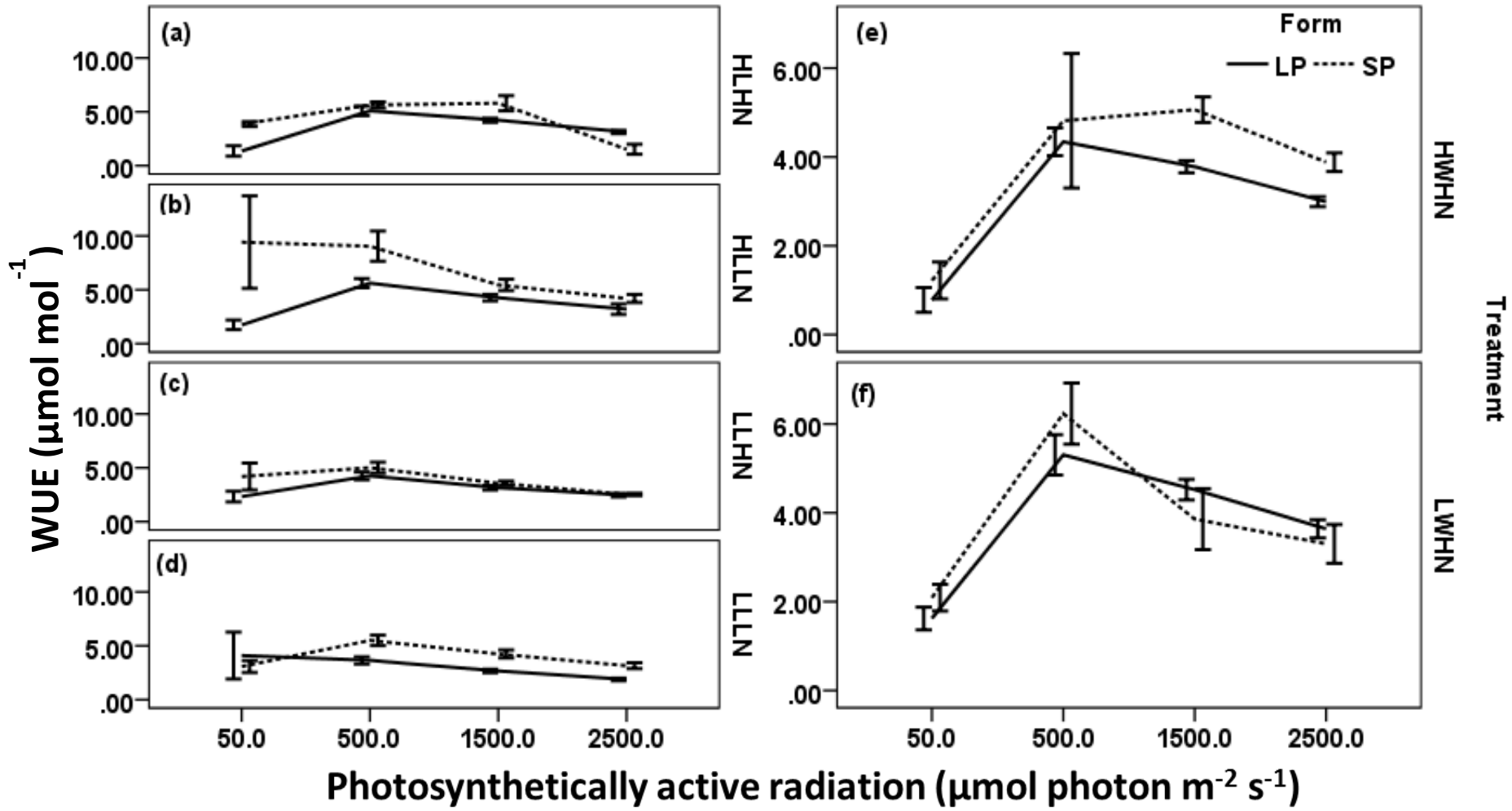


Figure 6.4. Change in water use efficiency (WUE) for LP and SP in response to incremental changes in PAR.

Table 6.2 Matrix of Pearsons correlation coefficients (r) for functional traits of LP and SP across different light, water and nutrient regimes. Significant correlations are shown by bold font and asterisks: ***$P \leq 0.001$, **$P \leq 0.01$ and $P \leq 0.05$ (n = 18-20)

LONG POD (LP)	**SLA**	**Total biomass**	**No. of tubers**	**BSD**	**WUE**	**ϕPSII**	**PNUE**	**Nmass**	**C:N**	**Amass**	**Amax**	**Chl.**
SLA	1											
Total biomass	-.403	1										
No. of tubers	-.073	.667**	1									
BSD	-.408	.941**	.516*	1								
WUE	-.383	.583**	.474*	.573**	1							
ϕPSII	-.030	.761**	.588**	.648**	.517*	1						
PNUE	-.570*	.352	.069	.360	.688**	.168	1					
Nmass	.588*	-.319	.404	-.442	-.472*	-.043	-.736**	1				
C:N	-.237	-.018	-.519*	.077	.318	-.112	.657**	-.924**	1			
Amass	-.107	.426	.715**	.311	.515*	.372	.552*	.157	-.215	1		
Amax	-.533*	.482*	.593**	.397	.595**	.341	.679**	-.126	-.108	.898**	1	
Chl.	.564*	-.498*	-.254	-.390	-.380	-.258	-.401	.568*	-.310	.034	-.239	1
SHORT POD (SP)												
SLA	1											
Total biomass	-.663**	1										
No. of tubers	-.519*	.893**	1									
BSD	-.702**	.768**	.602**	1								
WUE	-.424	.652**	.515*	.592**	1							
ϕPSII	-.411	.410	.310	.470*	.843**	1						
PNUE	-.213	.163	.161	.040	-.094	.068	1					
Nmass	.684**	-.471*	-.383	-.369	-.120	-.043	.097	1				
C:N	-.083	.039	.037	-.085	-.183	-.281	-.293	-.782**	1			
Amass	.392	-.142	-.069	-.174	-.117	.028	.659**	.812**	-.781**	1		

Amax	-.099	.111	.109	.108	.069	.226	.821**	.521*	-.785**	.877**	1	
Chl.	.027	.045	-.097	.008	.421	.481*	.314	.446	-.569**	.529*	.545*	1

It is evident that LP and SP responded differently under the high light and high nutrient scenario (HLHN; **Figure 6.3a)**, where carbon assimilation was significantly higher for SP during the low light of 50 and 500 µmol photon m^{-2} s^{-1}, then gradually decreased as PAR increased to 2500 µmol photon m^{-2} s^{-1}. In sharp contrast, LP began with low carbon assimilation rates at low light levels, and then gradually increased in response to an increase in light **(Figure 6.3a)**. Under the light experiments, change in A_{max} in response to PAR was not significantly different between LP and SP under HLLN, LLHN and LLLN treatments, although performance lines of SP were always above that of LP in the low light condition **(Figure 6.3b-d)**. Under the water experiments, SP showed a higher increase in $A_{\max}$ than LP in response to increase in PAR when there were high water and nutrient resources (HWHN; **Figure 6.3e)**. In contrast, there was no significant difference in the rate of change of A_{max} in response to PAR between LP and SP for the LWHN treatments **(Figure 6.3f)**.

LP and SP responded similarly in terms of WUE across treatments and PAR levels **(Figure 6.4a-d)**, although there was a significant effect of the interaction of treatment x PAR levels ($F_{5, 455}$ = 2.60, P<0.02). The significant interaction suggests that the response of each form varied depending on resource condition. Thus, SP appeared to be slightly more water use efficient at lower light levels (50, 500 µmol photon m^{-2} s^{-1}) and then becoming similar to that of LP for the remaining light levels for HLLN and HLHN treatments **(Figure 6.4e,f).**

Are there any differences in trait correlations between LP and SP?

Across all light, water and nutrient levels, there were significant trait correlations between total biomass and the following traits, SLA (r = -0.663), the number of tubers (r = 0.893), stem diameter (r = 0.768), WUE (r = 0.652) and mass based leaf N (r = -0.471) in SP **(Table 6.2)**. Traits significantly linked to total biomass in LP were area based $A_{\max}$, (r = 0.482) chlorophyll content (r = -0.496), effective quantum yield (ϕ_{PSII}) (r = 0.761), the number of tubers (r = 0.667), stem diameter (r = 0.941) and WUE (r = 0.583) **(Table 6.2 and Figure 6.5)**. PNUE, mass based Amax and C: N ratio were not significantly linked to total biomass in both LP and SP. It is interesting that SLA, a trait that facilitates photosynthetic capture was significantly linked to growth/biomass only in SP **(Figure 6.5c).**

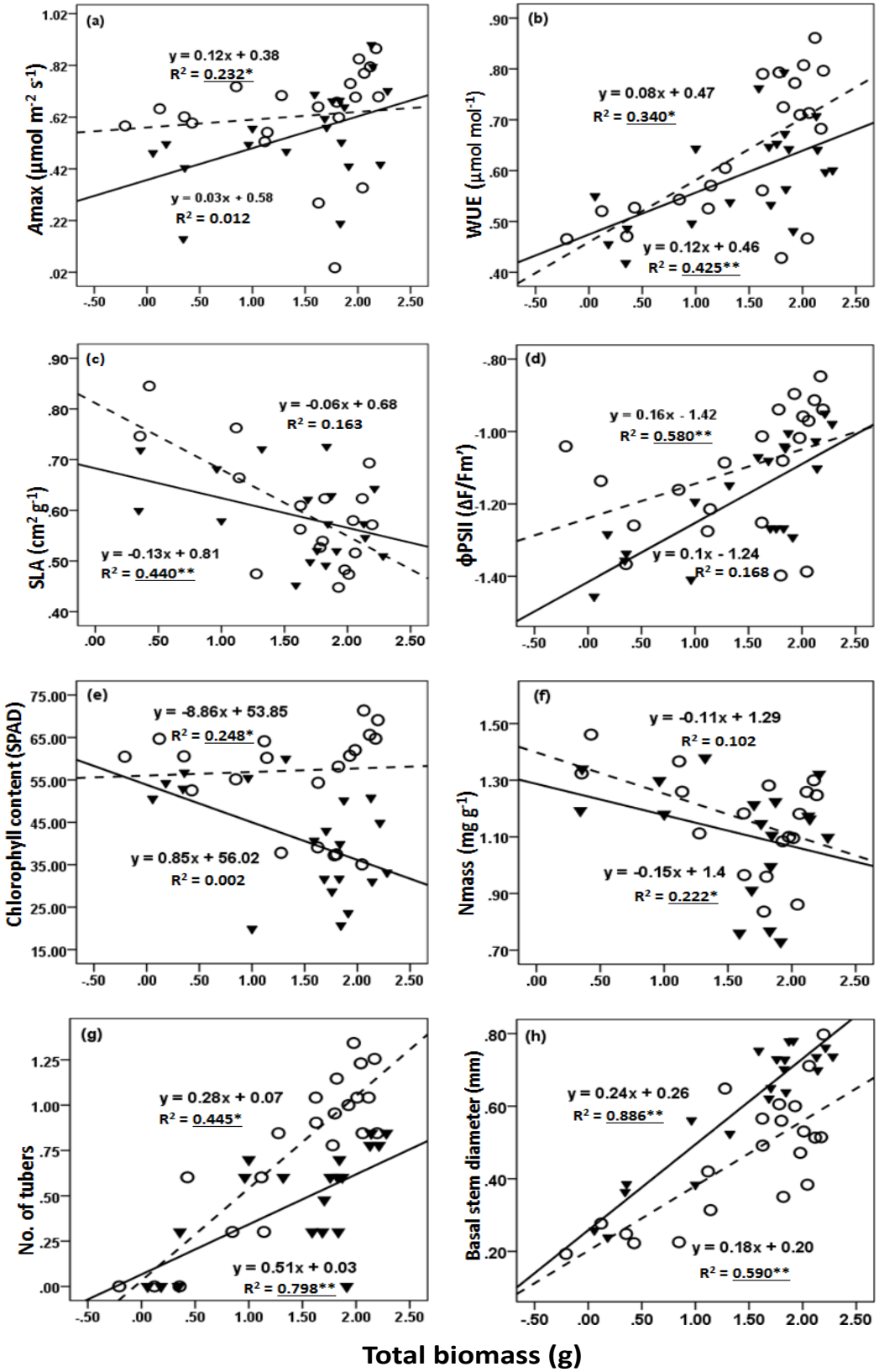


Figure 6.5. Relationships across light, water and nutrient regimes between total biomass accumulated vs. performance (number of tubers, basal stem diameter, SLA, Chlorophyll content) and physiological traits in LP (triangles, solid line, upper equation) and SP (open circle, dotted line and lower equation). Significant relations ($P<0.05$) are shown by underlined R^2 values, two stars (**) reflect significance at 99% while a single star (*) indicates significance at 95%.

Table 6.3. Correlation coefficients (r), and slope of performance traits (SLA and total biomass) against physiological traits for LP and SP in response to high light conditions, regardless of nutrient level. R values in bold are significant, two stars (**) reflect significance at 99% while a single star (*) indicates significance at 95%. (N ranged from 18 - 20 per bivariate relationship). The slope of the correlation equation gives an indication of plasticity of the bivariate trait relationship under the conditions considered (see Osunkoya *et al.* 2010, Table 4)

	SLA		**Total Biomass**	
	Correlation, r		Correlation, r	
	SP	LP	SP	LP
Plant level traits				
Total Biomass	0.359	-0.132	1	1
Shoot/root ratio	0.018	0.122	0.369	0.302
No. of tubers	273	-0.066	0.570**	0.713**
Basal stem diameter	-0.307	-0.234	0.493*	0.741**
Leaf level traits				
SLA	1	1	0.359	-0.132
A_{max}	0.054	-0.499	0.639**	0.528*
A_{mass}	0.483*	-0.109	0.593*	0.329
WUE	0.037	-0.25	0.153	-0.169
PNUE	0.146	-0.535*	0.427	-0.002
N_{area}	-0.092	0.079	0.294	0.218
N_{mass}	0.486*	0.5	0.464	0.109
Chl	-0.012	0.468	0.590*	0.071
ϕ_{PSII}	0.138	0.353	0.539*	634**
No of significant correlations	2	1	7	4

Table 6.4. Correlation coefficients (r) and slope of performance traits (SLA and total biomass) against physiological traits for LP and SP in response to high nutrient conditions, regardless of light and water level. Correlation (r) values in bold are significant, two stars (**) reflect significance at 99% while a single star (*) indicates significance at 95%. The slope of the correlation equation gives an indication of plasticity of the bivariate trait relationship under the conditions considered (see Osunkoya *et al*. 2010, Table 4)

	SLA		**Total Biomass**	
	Correlation, r		Correlation, r	
	SP	LP	SP	LP
Plant level traits				
Total Biomass	-0.474	-0.461	1	1
Shoot/root ratio	0.065	-0.125	0.029	0.269
No of tubers	-0.451	-0.365	0.591*	0.826**
BSD	-0.635	-0.432	0.628*	0.803**
Leaf level traits				
SLA	1	1	-0.474	-0.461
A_{max}	-0.442	-0.475	0.778**	0.603**
A_{mass}	0.533*	-0.12	0.304	0.472
WUE	-0.579	-0.441	0.833**	0.566*
PNUE	-329	-0.463	0.669**	0.595*
N_{area}	-0.496	0.067	0.569*	-0.014
N_{mass}	0.978**	0.892**	-0.381	-0.369
Chl.	-0.132	0.694**	0.528	-0.266
ϕ_{PSII}	-0.529	-0.217	0.905**	0.838**
No. of significant correlations	7	2	8	6

Considering all possible bivariate relationships for the 13 traits in the study (i.e. 78 pairwise comparisons), number of significant correlations were not different between LP (31/78) and SP (24/78) **(Table 6.2)**, although LP showed slightly more correlations. To test whether there was a shift in trait integration or coordination in response to high resources, some performance traits were correlated with SLA and total biomass for high light and high nutrients separately (**Tables 6.3 and 6.4**). In the high light conditions **(Table 6.3)**, more traits were significantly correlated with biomass gained for SP (7 out of 12) than LP (4 out of 12). Two physiological traits, A_{mass} and N_{mass} were significantly linked to SLA for SP but only one trait (PNUE) for LP.

In high nutrients, more traits (7 out of 12) were correlated with SLA for SP, being biomass gained (negative), basal stem diameter (negative), Amass (positive), WUE (negative), total leaf N (negative), N_{mass} (positive) and ϕ_{PSII} (negative). In contrast only two traits (N_{mass} and Chl.) were significantly and positively associated with SLA for LP. A higher number of traits (compared to the high light scenario) were linked to biomass gained for LP (6 out of 12), but more traits were still significantly correlated with total biomass for SP (8 out of 12) (**Table 6.4**). Overall, it is safe to assume that there is more trait integration in SP than in LP **(Tables 6.3 and 6.4, Figure 6.5).**

A graphical representation of a principal component analysis (PCA) of LP and SP based on 13 traits and four treatments (HLHN, HLLN, HWHN and LLHN) is shown in **Figure 6.6**. The other treatments were not included because some physiological and chemical traits were not measured in those treatments. The principal component analysis shows that the first two axes explained 60.6% of the total variation in the data. The first axis explained 33.23% of the data variation and was strongly correlated with RUE traits (WUE, PNUE), SLA and total biomass. The second axis explained 28.40% of the total variation in the data and was strongly linked to C:N ratio, shoot/root ratio, both area and mass based CO_2 assimilation rate and total leaf N per mass **(Figure 6.6)**. The two forms of *D. unguis-cati* clustered together on the first axis but were significantly separate along the second axis (see the separation shown by dotted shapes comparing the two forms under the treatments). The separation on the second axis was strongly driven by varying responses to treatments, especially HWHN, HLHN and HLLN. Axis III and IV were not shown because together they explained less variation in the data (11 and 10% respectively).

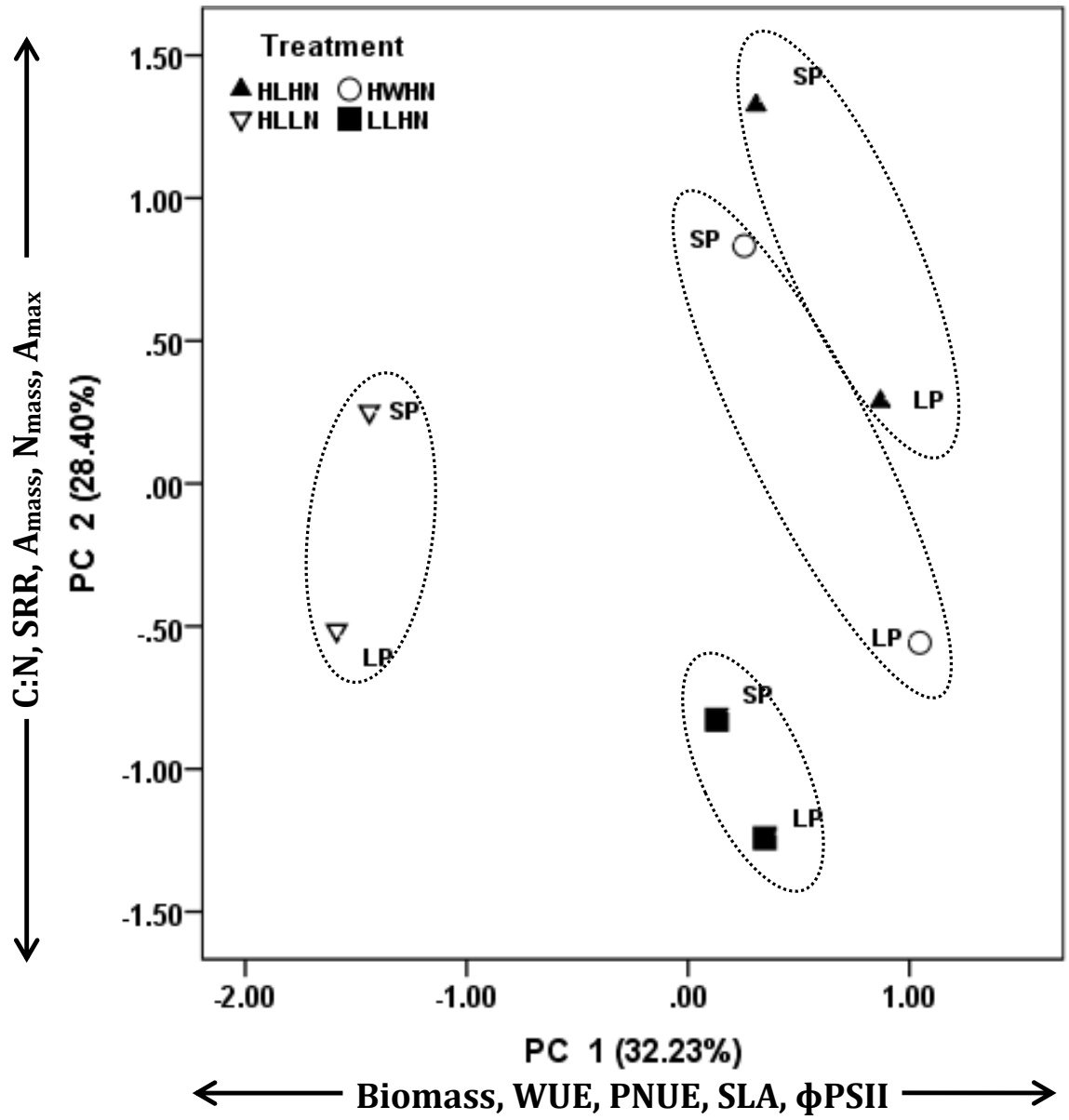


Figure 6.6. Principal component analysis of LP and SP across four treatments based on mean of 13 ecophysiological traits projected on the first two axes. The traits on each axis are the main drivers of the variation explained by that axis. The percentage of the variance explained by each principal component is shown in brackets. There was no determination of leaf chemistry for treatments that are not included in this PCA because of insufficient leaf materials. Dotted lines connect LP and SP under similar treatments for the sake of comparison.

6.5 Discussion

This study indicates similar responses to resources for some traits (e.g SLA, shoot/root ratio) under certain scenarios. Both forms performed well under high nutrients indicating some areas of commonalities. However, there are fundamental differences in physiological and performance traits in response to changes in light, water and nutrient resources by LP and SP. The physiological performance resulted in varying plant performances (indicated by biomass accumulation by 15 months of growth) in certain treatments, implying context dependent differences of the two forms in response to light, water and nutrient resources. Overall, SP appears to have an advantage over LP in performance and fitness traits response to resources. Increased nutrient enrichment resulted in a greater performance in both forms, but SP exhibited improved performance in the high light scenario. Thus, traits that were associated with SLA and total biomass accumulation in SP were high-resource dependent. Increased nutrients indicate disturbance (Hobbs and Huenneke 1992). In disturbed environments, species that are better able to exploit fluctuating resources will likely invade the system (Cordell *et al.* 1998; Leffler *et al.* 2014; van Kleunen *et al.* 2010b).

Biomass production and allocation patterns in response to resources

Biomass accumulation is often referred to as a performance (Luo *et al.* 2015) or fitness trait (Osunkoya *et al.* 2010a). This is because biomass production is closely linked with relative growth rate (RGR) in plants (Malhi *et al.* 2015). From this study, the direction of difference indicates that LP accumulated more biomass than SP in response to resources, but this was explained by LP's increased biomass production under the HLHN only. This result is in agreement with Taylor and Dhileepan (2012) who found that LP accumulated more biomass than SP in a field experiment that involved the application of extra nutrients to the soil. As disturbed sites are often characterised by high light and nutrient availability (Davis *et al.* 2000; Melbourne *et al.* 2007), this relationship could imply that LP is a better performer in disturbed environments. However, results from another glasshouse experiment found that SP accumulated more biomass under low nutrient scenarios (Chapter 5), a trend that the current study also found (see **Figure 1a**, treatment HLLN).

This study supports the context-dependent hypothesis (Moravcová *et al.* 2015) of trait differences as evidenced by significant interactions of form and treatments in explaining

biomass accumulation, tuber development and stem diameter differences between LP and SP. Burns (2004) compared three pairs of invasive versus closely related native species of family Commelinaceae and found that the invasive ones had higher biomass accumulation (RGR) only under high nutrient scenarios. However, there was no difference in RGR between invasive and native species under low nutrient conditions and both groups were equally plastic for this trait (Burns 2004). In our study, variation in response patterns to similar light, water and nutrient conditions by the two forms could imply fundamental differences in carbon economy (Osunkoya *et al.* 2010a). For vines like *D. unguis-cati* which do not self-support, accumulated biomass is used to develop elongated stems and branches that help increase light harvesting capability (Campanello *et al.* 2016; Putz 2005). SP has been demonstrated to have higher branching and faster stem elongation than LP under glasshouse conditions (see Chapter 5 of this thesis). We thus argue that, when evaluating performance of vines which do not support themselves structurally, more attention should be given to stem length and branching dynamics.

In this study, tuber development was more strongly correlated with total biomass accumulation in SP across low and high resource conditions. SP developed a significantly higher number of tubers than LP, which could have contributed to a reduced shoot/root ratio in SP. Tubers act as a sink or storage organs for moisture and photo-assimilates, and they may also regenerate producing new plants (Janeček and Klimešová 2014; Orthen 2001; Schubert and Feuerle 1997). Previous investigations of plant development over time between LP and SP under similar conditions found SP to produce more tubers early in development than LP. Apart from seed germination (Vivian-Smith and Panetta 2004b), *D. unguis-cati* also propagates vegetatively through tubers (Downey and Turnbull 2007; Osunkoya *et al.* 2009). Horizontal stems and branches trailing along the ground develop adventitious roots at nodes (Vaughn and Bowling 2011), which penetrate the soil and develop tubers (Osunkoya *et al.* 2009). If new plants regenerating at the nodal tubers are severed from the mother plant, they grow independently as genets (Osunkoya *et al.* 2009). Tubers can also remain dormant for extended periods belowground as a stress tolerance strategy (Orthen 2001). Thus, this finding of greater linkage between tuber density and biomass gained for SP suggests a greater niche pre-emption and dominance of invaded landscape for this form (Ashton *et al.* 2010).

This study also indicates that SP exhibited lower shoot/root ratio in more low resource treatments than LP in the ratio 3:1. Lower shoot/root ratios are often associated with species that are adapted to low resource environments, which invests more belowground to potentially

maximise nutrient acquisition (Drenovsky *et al.* 2008). Low shoot/root ratio is a trait that has been linked to fast growing invasive species (Funk and Vitousek 2007; Rajaniemi and Reynolds 2004).

Leaf traits in response to light, water and nutrients

LP is known to have broader leaves than SP (Shortus and Dhileepan 2011), and this study confirms this trend in that LP had significantly higher leaf area (LA) than SP in response to light, water and nutrient resources. However, SP invests more biomass in the leaves, and this is shown by similar specific leaf area (SLA = LA/Leaf dry mass) between LP and SP under the same treatments. High specific leaf area (SLA) is an important plant trait that facilitates capture of photosynthetically active radiation (PAR) and is often associated with invasive species (Grotkopp and Rejmánek 2007), but see Garcia-Serrano *et al.* (2005). Higher SLA indicates thinner leaves, which are cheaper to produce quickly compared to thicker leaves for the same surface area (Poorter and Remkes 1990).

With thinner and broader leaves, LP appears to perform better than SP in this regard. Heavy investment in constructing leaf tissue resulting in thicker leaves (e.g. by SP in this study) (Lambers and Poorter 1992) is a trait often associated with slow growing plants (van Kleunen *et al.* 2010a). Considering SP to be a more successful colonizer than LP based on current abundance rates, our study does not appear to associate SLA with colonization success. However, developing thicker leaves maybe a strategy to compensate for less surface area to increase photosynthetic apparatus (palisade parenchyma) in SP. Indeed, SP has been found to have significantly thicker palisade mesophyll tissue than LP (see Chapter 3 of this thesis). Baars and Kelly (1996) observed that the most likely vines to be successful colonizers are those that are adaptable to low light conditions. Using five vines (3 invasive versus 2 native species) from New Zealand, they found that invasive vines were characterised by a high degree of shade tolerance but also had ability to grow rapidly under high light situations (Baars and Kelly 1996). *Monstera gigantea*, a tropical vine was found to grow towards shaded places created by tree canopies (a type of negative phototropism called skototropism) (Strong Jr and Ray Jr 1975) in order to locate vertical supporting structures (Vaughn and Bowling 2011). The two forms respond to low light conditions by investing more biomass per unit area on their leaves, indicated by higher SLA under LLHN than HLHN treatments for both forms.

Physiological responses to light, water and nutrient resources

SP exhibited higher values than LP for the majority of physiological traits overall, indicating differences in resource acquisition and use between the two forms. In the low nutrient scenario, carbon assimilation was twofold higher under low light when compared to high light for the SP form. This was accompanied by a greater leaf N concentration at low light levels. The leaf economic spectrum suggests that high Amax needs more concentrations of leaf N to drive rapid growth (Wright *et al.* 2004). Higher Amax under low light conditions could be a strategy by SP to increase growth to reach greater heights for more light acquisition. Faster growing plants have a large demand for nutrients, thus low C: N ratios (Luo *et al.* 2015).

Across the various treatments, there were no differences in resource use efficiency (RUE), measured in this study as photosynthetic nitrogen use (PNUE), suggesting that LP and SP use resources in a similar way, at least under similar light, water and nutrient resources. When light conditions are considered separately, a negative relationship between PNUE and biomass gained was obtained for LP - an indication of less RUE in this form. However, considering nutrient conditions separately, both forms show a positive relationship between PNUE and biomass gained. However, correlation coefficient (r) values, as well as the relationship slope, were greater in SP suggesting that at a given PNUE, a higher biomass was always attained for SP relative to LP and indicating less RUE in the LP form.

Previously, studies have found non-native invasive species to have higher RUE than native non-invasive congeners (Firn *et al.* 2012; Funk and Vitousek 2007). In the high light scenario, only SP showed positive (albeit marginally significant) relationship between PNUE and biomass gained, while in LP the relationship was a flat line, again an indication of better RUE of SP relative to LP (Osunkoya *et al.* 2010b). However, Funk (2008) argues that traits such as PNUE and WUE may not correlate with fitness measures on a short time scale such as the current study, which was little over a year or may reflect a context-dependent of the traits as shown in this study. On the whole, SP exhibited better WUE than LP. However, this trait was significantly associated with biomass accumulation in both LP and SP, and therefore not informative in differentiating the two forms.

Physiological response to increasing PAR

Physiological traits of LP and SP respond to short-term changes in PAR in a similar fashion, except for plants grown in high light and increased nutrient resources (HLHN treatment). At low light levels, SP shows better performance in net CO_2 assimilation (Amax) but shows a decrease in this trait value with increasing PAR. The implication is that SP performs better under low light scenarios such as understory while there is an interruption of its photosynthetic activity at higher PAR. This downregulation of photosynthetic activity at high PAR could be a result of photoinhibition and photooxidation (Powles 1984), which usually occurs when shade plants are exposed to excessive light (Van Goethem *et al.* 2015). Vines that are successful invaders are known to be adapted to low light habitats such as forest understories (Baars and Kelly 1996; Granados and Körner 2002). A better physiological performance by SP in low light indicates that this form requires minimum or no canopy disturbance to establish and grow. **Figure 4** also shows that SP performs better still at moderate light conditions of approximately 1000 µmol photon m^{-2} s^{-1}. This suggests that this form would still be able to exploit canopy disturbances to colonize forests. It has been suggested that vines that are successful colonizers are adapted to shade conditions but grow rapidly as well in high light (Granados and Körner 2002; Teramura *et al.* 1991).

On the other hand, LP started off with a constrained photosynthetic activity at low PAR of 50 and 500 µmol photon m^{-2} s^{-1}, then increase markedly in response to increasing PAR, indicating a better use of excess photon fluxes which results in fixing more carbon. This could explain the higher performance of LP by producing more biomass under HLHN. Considering the occurrence of these two forms in riparian and rainforest habitats that have canopies blocking sunlight, it would mean that SP would perform better under those circumstances. LP would perform better under disturbed open spaces, with high light resources. LP may therefore be less of a threat to larger remnant forests with lower degree of canopy disturbance because it requires mostly high light to grow. Canopy gaps from tree clearing promote invasion by creepers and vines (Gatti *et al.* 2015; Schnitzer and Bongers 2002). This result is significant in that not only does it imply the greater potential for invasiveness by LP, but also suggests slight differences in light capture strategies between the two forms. In a disturbance related scenario of high water and high nutrients, SP has a higher increase in A_{max} than LP in response to increases in PAR. This implies that SP responds better when there is an interaction of high water and nutrient resources, which also resulted in a marginally higher biomass than LP. SP also shows slightly higher WUE than LP in response to high PAR (1500 and 2500 µmol photon m^{-2} s^{-1}) but not PNUE.

Trait coordination and ordination in response to resources

The traits measured in this study were correlated for each form to assess the extent of covariance among them, which gives an indication of phenotypic integration (Luo *et al.* 2015; Osunkoya *et al.* 2014). Consistent with Luo *et al.* (2015), we did not find any significant differences in trait correlations when considering all possible interactions in the study. This relationship could indicate similar phenotypic integration for the two forms. However, SLA was significantly linked to total biomass accumulation in SP but not LP. There was a significant shift of trait integration in favour of SP in response to high light and nutrients resources. Here, consistent with Osunkoya *et al.* (2010b), the results show that the strength of trait correlation was higher for SP than LP, in response to the high resource conditions. This means that SP exhibited a higher level of phenotypic integration than LP in response to elevated resources. Some previous studies have suggested that when traits respond to environmental fluctuations in a coordinated fashion, it enhances performance (Reich *et al.* 2003; van Kleunen and Fischer 2005). A well-coordinated response to environmental heterogeneity enables the plant to adapt better to changes (Luo *et al.* 2015; Osunkoya *et al.* 2010b; Osunkoya *et al.* 2014).

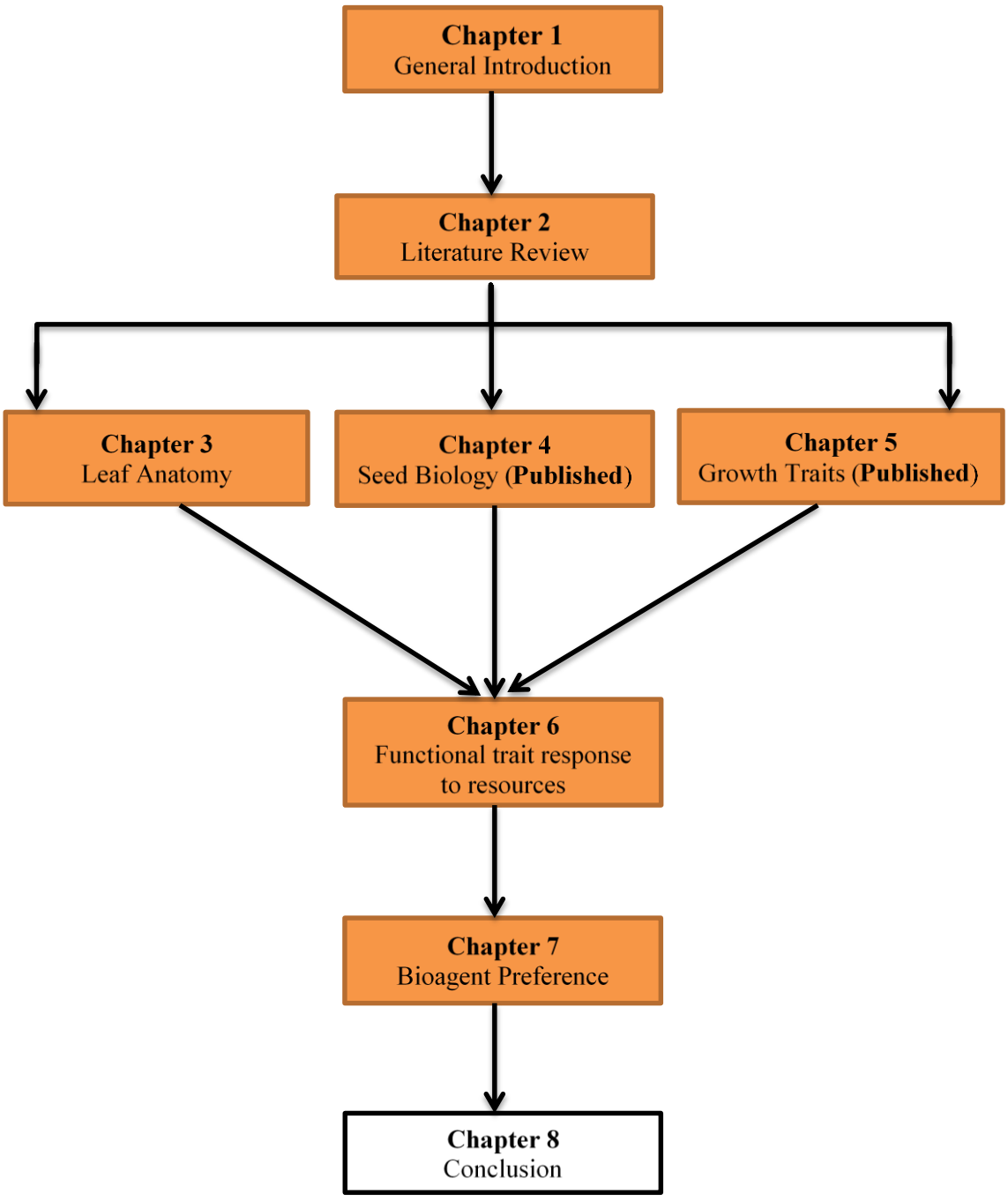
6.6 Conclusion

Overall, there was a better performance of SP in response to high light and nutrient conditions by way of exhibiting greater trait coordination. Regarding biomass accumulation, LP performs better in high light resources while SP performs better in low resource conditions. The theory of fluctuating resource availability holds that species that can exploit excess resources successfully colonize disturbed habitats (Davis *et al.* 2000). From a performance perspective, our results partly concur with Taylor and Dhileepan (2012) that LP has a potential of further spread. However, adaptation to low resources has been shown to be a trait of some invasive species (Funk and Vitousek 2007). Therefore, our results imply that SP would perform better and colonize low resource habitats. The study suggests that SP is more efficient in resource acquisition and use than LP.

The two forms seem to be placed slightly apart in terms of carbon economy and thus, would occupy slightly different positions in the LES (Wright *et al.* 2004), with SP positioned more towards the faster growing, high return on investment end (Penuelas *et al.* 2010). A strong

trait correlation in SP shows that this form exhibits a higher level of phenotypic integration than LP. High phenotypic integration (Luo *et al.* 2015) or coordination (Osunkoya *et al.* 2014) is a trait associated with invasiveness. Phenotypic integration is considered a phenomenon that could constrain non-adaptive phenotypic plasticity in plants (Pigliucci 2003), thus increasing fitness in heterogenous environments (Wanderley *et al.* 2016). LP and SP are separated along the second axis in the ordination space of a PCA, indicating varying patterns resource acquisition biomass allocation to roots and shoots and carbon assimilation in response to treatments.

The results from this study shed light on the differences that occur between LP and SP, adding to the growing prospectus that may eventually explain why the two forms have different prevalence in Australia. However, caution should be observed in extrapolating these results because experiments were carried out in a glasshouse that could not completely simulate the multiple ecological interactions that take place in natural environments. Residence times of LP and SP in Australia are also unknown, but they could have implications in the prevalence of the two forms (also see Pyšek *et al.* 2015).


Chapter 1
General Introduction
Chapter 2
Literature Review
Chapter 3
Leaf Anatomy
Chapter 4
Seed Biology (Published)
Chapter 5
Growth Traits (Published)
Chapter 6
Functional trait response
to resources
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Bioagent Preference
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Chapter 7: Do biological agents have any preferences for either form of *D. unguis-cati?*

7.1 Abstract

Biological control is the preferred management approach against the invasive vine, *Dolichandra unguis-cati* in Australia. The biological control programme for this species started in 2001 and so far, three biological control agents have been released. The occurrence of two morphologically distinct forms of this weed could potentially jeopardise the effectiveness of biological control strategies if the agents have a preference for either form. The aim of the study presented in this chapter was to use two of the released agents, *Hylaeogena jureceki* and *Carvalhotingis visenda* to assess whether they show a preference for any of the two forms. Because agents (or insects) feeding behaviour can be affected by resource availability, preference was also evaluated for plants grown under different levels of water and nutrients. Results show that *C. visenda* does not have a preference for any form while *H. jureceki* have a preference for long pod over short pod. Resource level had a significant effect on preference for both forms, with agents choosing the high nutrient plants the most. Lack of preference for either form by *C. visenda* implies that this agent is suitable for continual use against long pod and short pod. On the contrary, preference for long pod by *H. jureceki* might imply a potential lack of efficacy of this agent on short pod infestations. More research needs to be carried out in the field to substantiate findings from this study. An evaluation of biological control method against *D. unguis-cati* is suggested, especially in light of occurrence of long pod and short pod.

7.2 Introduction

Chemical and mechanical control options for *D. unguis-cati* are available but are often not used due to the sensitive ecosystems (riparian vegetation and rainforest) where it occurs (Dhileepan 2012). The need to apply these controls repeatedly over many years severely limits the size of infestations that can be treated. Hence, biological control is considered the most

desirable option to manage this invasive species. The biological control programme for *D. unguis-cati* began in 2001 with surveys in the native range of this species, especially Brazil, Argentina, Paraguay, Venezuela and Trinidad. These surveys identified nine insects as potential agents for the control of *D. unguis-cati* in the introduced range (Dhileepan *et al.* 2005; Sparks 1999). So far only three agents have been approved for release in Australia after host-specificity tests (Dhileepan *et al.* 2007a; Dhileepan *et al.* 2013; Dhileepan *et al.* 2007b). These are a leaf-sucking tingid, *Carvalhotingis visenda* Drake & Hambleton (Hemiptera: Tingidae) (Dhileepan *et al.* 2007b), a leaf tying moth, *Hypocosmia pyrochroma* Jones (Lepidoptera; Pyramidal) (Dhileepan *et al.* 2007a) and a leaf mining jewel beetle, *Hylaeogena jureceki* Oberberger (Coleoptera: Buprestidae) (Dhileepan *et al.* 2013). The effectiveness of various biological control agents on the two forms of *D. unguis-cati* is unknown. In the past, biological control agents have shown marked preference or avoidance to species with high intraspecific diversity such as lantana (Cilliers and Neser 1991; Zalucki *et al.* 2007).

Fluctuation of resources such as water and nutrients affect plant growth regimes and resource allocation of plants (Osunkoya *et al.* 2010a). The insect-performance hypothesis proposed by Larsson (1989) suggests that performance of some insects increases with increase in plant stress. Hsiao (1973) has extensively documented the effects of water deficit on different physiological and anatomical processes of plants, which may affect plant-herbivore interactions, directly affecting biological control (Müller-Schärer *et al.* 2004). The plant water stress hypothesis suggests that increased insect performance during plant water stress may be attributed to increased foliar nitrogen level (Huberty and Denno 2004). Thus, the resource-enemy release hypothesis (R-ERH) predicts that high resource plant species are likely to be susceptible to herbivory because they have high tissue nutrients that are consumed by insects (Blumenthal 2006). The R-ERH hypothesis combines the enemy release hypothesis (Keane and Crawley 2002) and the resource hypothesis of habitat invasibility (Davis *et al.* 2000). According to these hypotheses, the efficacy of biological control agents on invasive species may be affected by resource availability (Blumenthal *et al.* 2009). This underscores the need to test the preference of biological control agents under varying levels of resources using both forms of *D. unguis-cati* (or different genotypes of high intraspecific diversity in the weed).

At times, the lack of preference or lack of inadequate performance of biological control agents to the target weed may be due to the wrong choice of biological control agents or releasing them on the wrong plant species (Myers 2000). It could also be a result of adaptive

changes in the target plant in the introduced range (Müller-Schärer *et al.* 2004) or climate differences that may inhibit establishment of agents in the new environment (Gassmann and Schroeder 1995; Myers 2000). For example, failure of biological agents to establish in the leafy and Cypress spurges, *Euphorbia esula* L. and *Euphorbia cyparissias* L. was a result of morphological variations between spurges from the native and introduced range (Gassmann and Schroeder 1995). Biological control agents that developed on the leafy spurge in the native range were found to be incompatible with the spurges in the introduced range. Just like leafy spurge, *D. unguis-cati* (see Boyne *et al.* 2013a) is complex with variable forms in its native and introduced range.

No comparative study has been conducted to date to determine the preference of introduced agents on the forms of *D. unguis-cati.* The two questions that this study sought to answer were, 1) do the biological control agents currently used against *D. unguis-cati* in Australia have the same level of preference for the two morphologically distinct forms? 2) Does water and nutrient resource level affect the preference of biological control agents for the two forms?

7.3 Materials and Methods

Plants that were used in this study were obtained from seed germination trials described in Chapter 4 of this thesis (Buru *et al.* 2016). Two week old seedlings were transferred into plastic pots (dimensions: Width=200 mm, Height=190 mm, Length=200 mm) filled with locally available commercial seed raising potting mix containing trace elements (Osmocote). The seedlings were grown at the Ecosciences Precinct glasshouse facilities, Boggo Road, Dutton Park, Queensland (GPS coordinates: 27°29’41.5248’’ S; 153°1’49.2132” E) in Brisbane, Australia. Plants were watered once a day. Additional nutrients not supplied for next three months. After three months, plants were assigned into one of the following treatments:

1. Control: Plants in the control treatments were watered twice a week with no additional nutrients added.
2. High water (HW): Plants in this treatment were watered daily to pot capacity with no additional nutrients. See Chapter 6 on pot capacity.

3. Low water (LW): Plants in this treatment were watered once every two weeks with no additional nutrients.
4. High nutrients (HN) - Potting mix enriched with nutrients by adding granules of a slow-release all purpose fertiliser (Osmocote, NPK 21:2:6 plus trace elements) once every week. Plants in this treatment were also watered twice every week.
5. Low nutrients (LW): Plants in this treatment were watered twice a week with no additional nutrients

Preference tests commenced after the experimental plants had been subjected to different treatments for 2 months using two biological control agents that have been approved for release to control *D. unguis-cati* in Australia. These agents are a leaf-sucking tingid, *C. visenda* (Dhileepan *et al.* 2007b) and a leaf mining jewel beetle, *H. jureceki* (Dhileepan *et al.* 2013).

Tingids have an estimated generation time of 38 days, and both the adults and nymphs feed by sucking leaf sap causing chlorosis. Adult females produce more than 180 eggs in their life span (Williams 2003). Many female tingids normally lay their eggs in groups on the adaxial side of the leaves along the main veins. Nymphs emerge about 15 days after oviposition and undergo five nymphal instar stages for another 15 days (Dhileepan *et al.* 2007b).

Adult jewel beetles feed on leaves, preferring the young and tender leaves, and over time, they can cause a complete defoliation of plants. It takes newly emerged jewel beetle adults between 11-20 days to start oviposition, and their total lifespan is an average of 51.6 ± 4.6 days (Dhileepan *et al.* 2013). Eggs hatch into larvae that complete three instars while burrowing through the leaf lamina (Williams *et al.* 2008), eventually building a circular pupation disk at maturity (Snow and Kunjithapatham 2013). Pupation lasts up to 11 days before adults emerge and adults can live up to nine months (Williams *et al.* 2008).

Biological control preference experiments

Preference tests were carried out in insect-proof cages (dimensions: Length = 44cm, Width = 42 cm and Height = 86 cm) using unsexed adults due to the difficulty in sexing the biological control agents visually (see Dhileepan *et al.* 2013). For both biological control agents, preference tests were performed on LP and SP forms under control, water, and nutrient treatments. Under the control conditions, two plants of each form (4 plants in total) were evenly placed in a cage and 20 individual agents released in the middle of the cage. Under the water

and nutrient treatments, single plants of each form from both HW and LW (or HN and LN) respectively (a total of 4 plants), were placed in the cages (Table 7.1). Likewise, 20 agents were released in the middle of the cages. Biological control agents were left for two days to acclimate to the cage environment before the start of data collection. The number of agents per plant (choice = preference) was recorded every day, at about the same time (between 12 pm – 2 pm) for five days. Plants in the cage were moved around daily to reduce the location effect on the choice of agents. After day 5, the number of damaged leaves and oviposition per replicate were recorded. For each treatment, the above process was replicated five times, each time with a new set of plants and agents.

Table 7.1 Number of plants of LP and SP in each replicate for preference tests involving control plants (1), nutrient treatments (2) and water treatments (3)

Preference Test	Treatment	**Replicate or Cage**	
		LP	**SP**
1	**Control**	2 plants	2 plants
2	**Nutrients**	1 HN 1 LN	1HN 1LN
3	**Water**	1 HW 1 LW	1 HW 1 LW

Statistical analysis

Data were tested for normality and whether they violated assumptions of homogeneity of variances using Levene's test of equality of error variances. All data were found to violate assumptions of homogeneity of variances and were square root transformed before analysis. A two-way factorial (repeated) measurements design analysis of variance (ANOVA) was used to determine the effects of the form (LP/SP) and treatment (HW, LW, HN, LN and Control) on the choice of biological control agents per plant, leaf damage and oviposition. Where treatments were found to significantly affect the preference of agents, a Tukey LSD post-hoc

statistic was used to compare specific means. Statistical analyses were performed using SPSS (version IBM SPSS Statistics 21).

7.4 Results

An overall two-way repeated ANOVA for the combined data of the jewel beetle and the tingid shows that form does not have an effect on the preference of the agents ($F_{1, 80} = 1.976$; $P = 0.164$). Preference is better explained by treatment ($F_{1, 80} = 10.198$; $P = 0.001$) followed by differentiation of the biological control agent ($F_{1, 80} = 4.923$; $P = 0.029$) but no interactions **(see Table 7.1 and Table 7.2).**

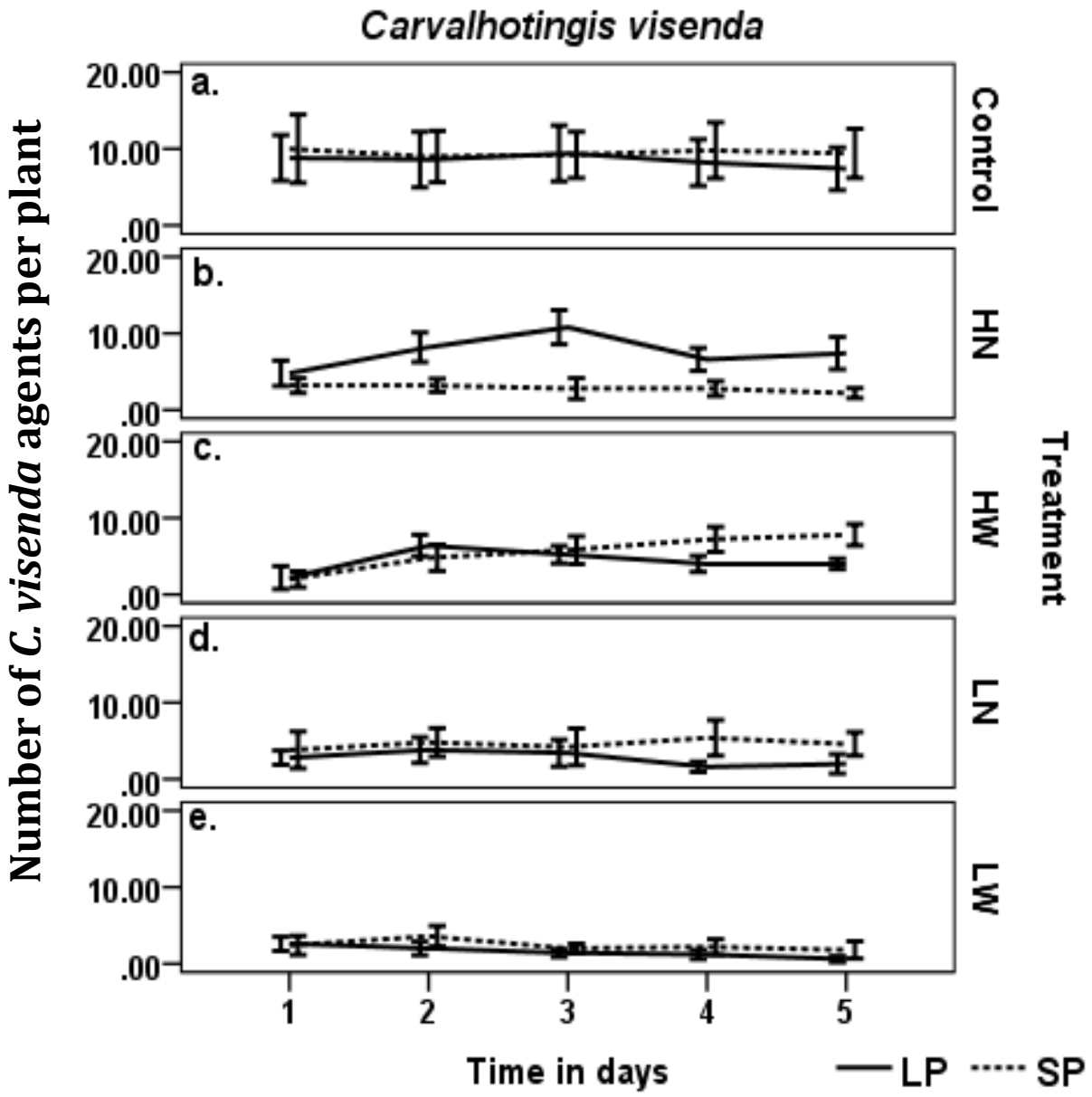


Figure 7.1. Mean number (± SE) of biological control agents found on the two forms of *D. unguis-cati* over time under different water and nutrient treatments. HN: High nutrients; LN: Low nutrients; HW: High water and LW: Low water.

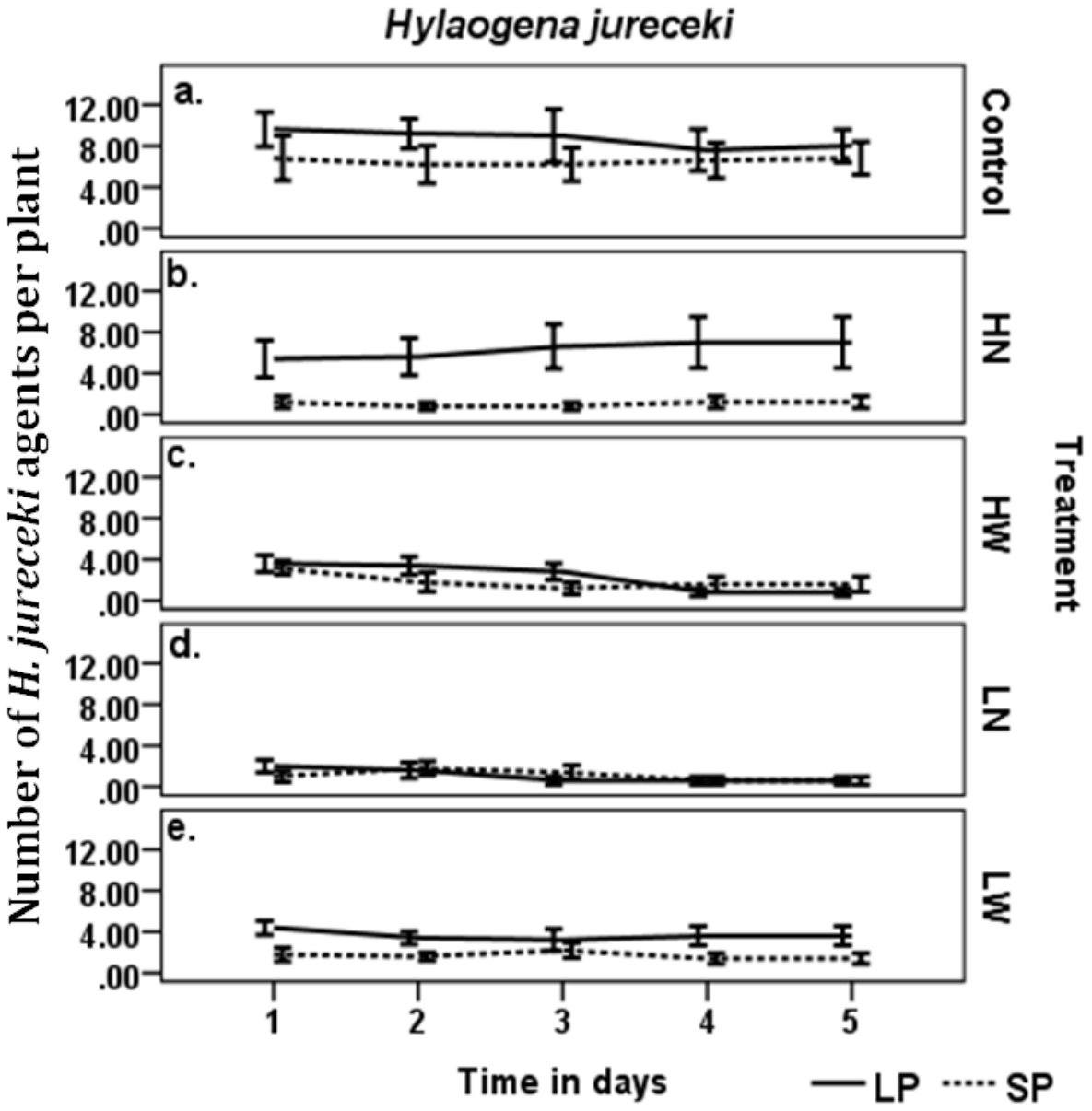


Figure 7.2. Mean number (± SE) of biological control agents found on the two forms of *D. unguis-cati* over time under different water and nutrient treatments. HN: High nutrients; LN: Low nutrients; HW: High water and LW: Low water.

Leaf sucking tingid (Carvalhotingis visenda)

The tingid does not appear to have any preferential attraction to either of the two forms of *D. unguis-cati* in our study ($F_{1, 40}$ = 0.001; P = 0.984). Preference to the forms is only marginally driven by the treatment under which the plants were growing ($F_{4, 40}$ = 3.826; P= 0.010; **Table 7.1**). Tingids show slight but not significant preference for the LP form growing under a high nutrient availability situation (**Figure 7.1d**), followed by those growing under high water (**Figure 7.1c**). In the well-watered plants (HW) however, more tingids seem to get attracted to SP plants towards the end of the trial (**Figure 7.1c**). In general, plants of both forms growing under stressful low resource conditions (LN, LW) attracted fewer tingids (**Figure 7.1e**).

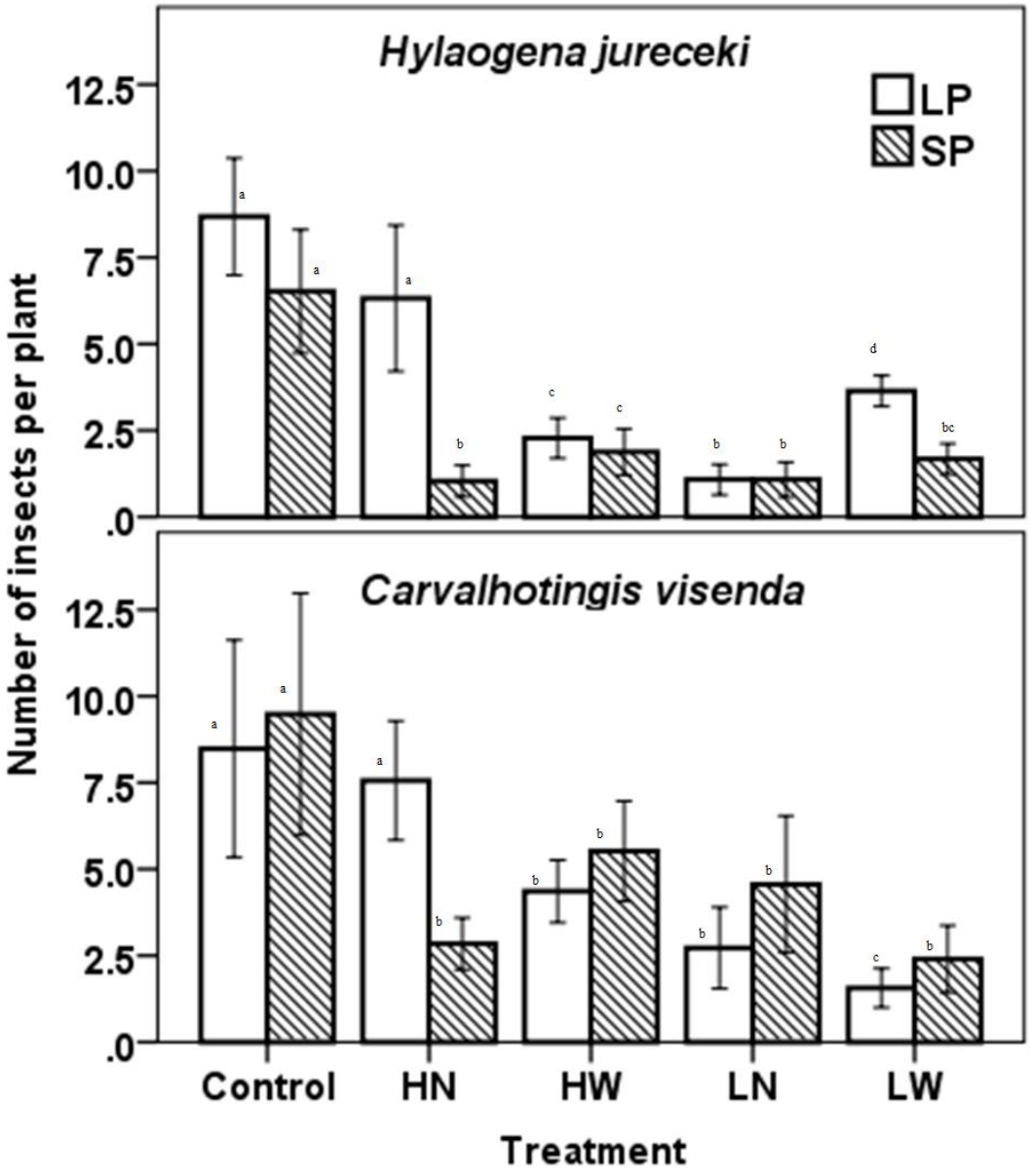


Figure 7.3. The number of biological control agents (± SE) on both forms of *D. unguis-cati* at the end of the experiment. HN: High nutrients; LN: Low nutrients; HW: High water and LW: Low water. Similar letters above the bars indicate insignificant differences between forms and treatments.

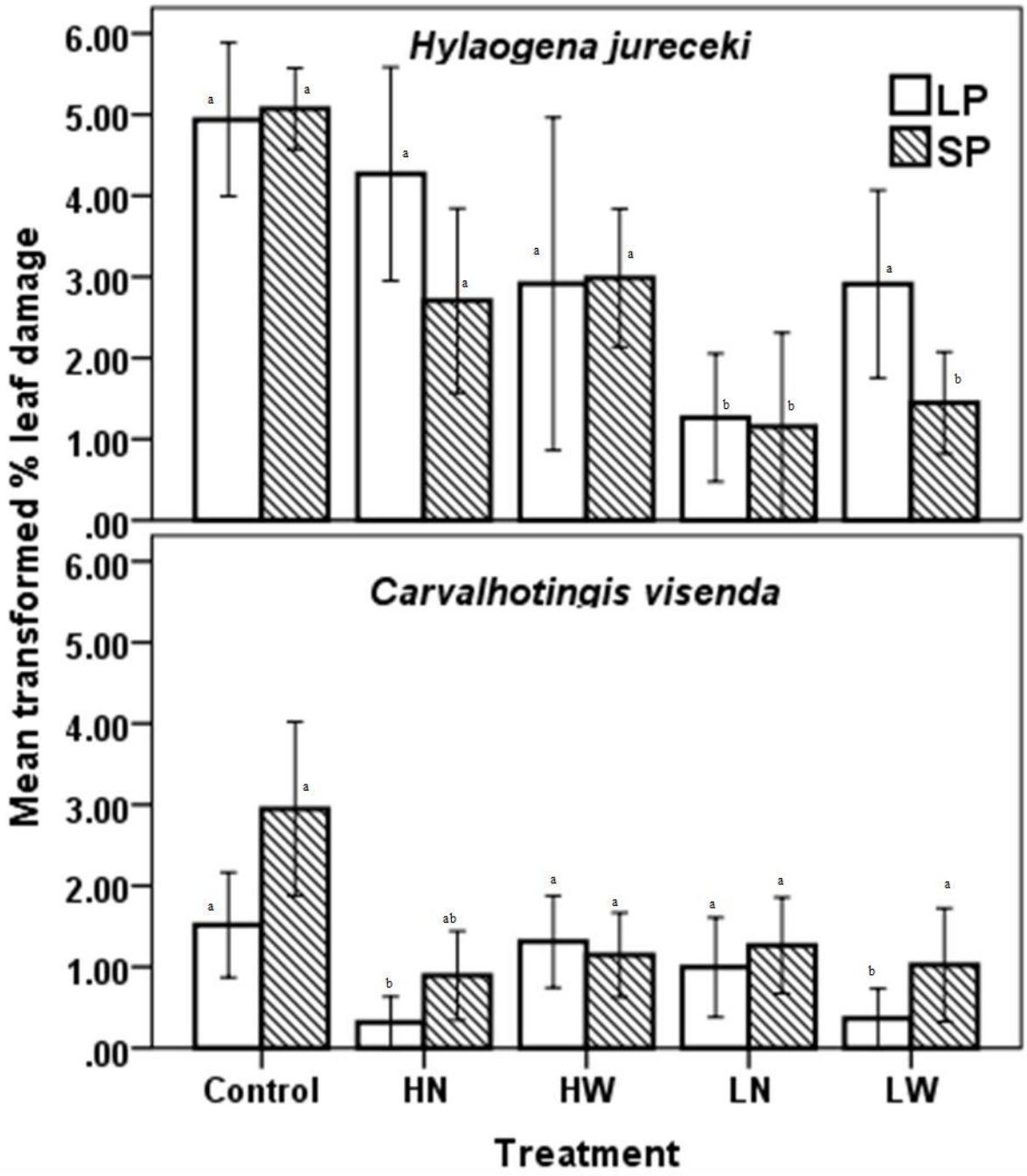


Figure 7.4. Square-root transformed percentage of leaf damage (± SE) caused by biological control agents on plants growing in different water and nutrient treatments. Similar letters above the bars indicate insignificant differences between forms and treatments

Leaf mining beetle (Hylaogena jureceki)

Overall, a repeated ANOVA shows that the jewel beetles preferred LP to the SP ($F_{1, 40}$ = 7.779; P = 0.008; **Table 7.1**). Treatment had a significant effect on the number of jewel beetles choosing a particular form ($F_{1, 40}$ = 10.282; P = 0.0001) but there was no interaction between form and treatment ($F_{1, 40}$ = 1.755; P = 0.157). More beetles were attracted to the LP plants under higher nutrient resources, but this trend was not observed during the high water scenario **(Fig. 2b,c)**. However, under low water (LW), still LP appears to attract slightly more beetles than SP, although not statistically different (**Figure 7.2e**). The jewel beetles prefer LP than SP under the control scenario, although the difference is not statistically significant **(Figure 7.2)**. Other factors beyond the scope of the study could have been responsible for the trend.

A summary of the preference of biological control agents at the end of the experiments appears to suggest that more beetles preferred the LP while more tingids chose SP (**Figure 7.3**), with the exception of the high nutrients treatments (HN).

Leaf damage and oviposition

Overall, there is no significant difference between LP and SP regarding leaf damage caused by the two biological control agents (**Figure 7.4, Table 7.2**) and there is no interaction of fixed effects (**Table 7.2**). Leaf feeding damages by the two agents cannot be compared because the nature of their leaf damage is different. The jewel beetle is a leaf miner while tingid sucks the leaf sap. Oviposition by the jewel beetle is not consistent in its response to the main effects as evidenced by a significant interaction of form and treatment ($F_{1, 40}$ = 15.657; P < 0.001). On the other hand, form does not have a significant effect on the tingid oviposition ($F_{1, 40}$ = 0.168; P = 0.737), but oviposition variation is only driven by treatment ($F_{1, 40}$ = 4.575; P < 0.004) (**Table 7.2**).

Table 7.2 Results of a two-way repeated ANOVA of preference (number of biological control agents per plant), leaf feeding damage and oviposition of two biological control agents with fixed effects structure of form (LP/SP) and treatments (HW, LW, HN, LN and Control)

Sources of variation	**Jewel beetle (*Hylaogena jureceki*)**							**Tingid (*Carvalhotingis visenda*)**					
		Preference		Leaf damage		Oviposition		Preference		Leaf damage		Oviposition	
	df	*F*-ratio	*P*-value	*F*-ratio	*P*-value	F-ratio	*P*-value	F-ratio	*P*-value	F-ratio	*P*-value	F-ratio	*P*-value
Form	1	7.779	0.008	0.673	0.417	8.782	0.005	0.001	0.984	1.967	0.168	0.115	0.737
Treatment	4	10.282	0.0001	3.176	0.23	15.657	0.0001	3.826	0.010	2.148	0.093	4.575	0.004
Form*Treatment	4	1.755	0.157	0.284	0.887	9.866	0.0001	1.023	0.407	0.440	.779	0.856	0.498
Error	40												
Total	50												

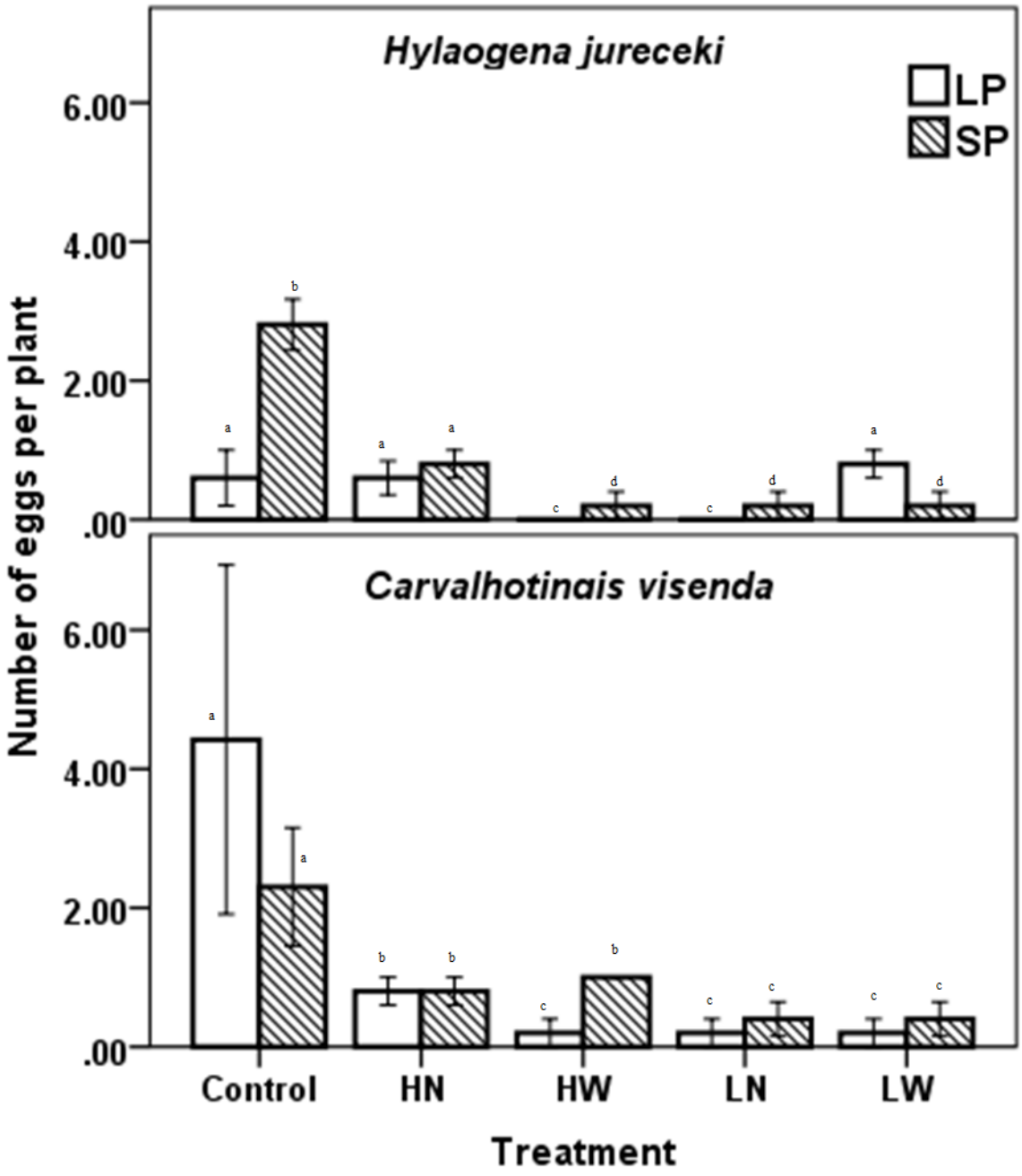


Figure 7.5. Square root transformed estimates of oviposition (± SE) by biological control agents on LP and SP growing in different treatments. Similar letters above the bars indicate insignificant differences between forms and treatments

7.5 Discussion

To the best of our knowledge, this is the first study to test the preference of biological control agents to the two forms of *D. unguis-cati* in Australia. The preference of *C. visenda* and *H. jureceki* for either the LP or SP form was evaluated by their choice for feeding and oviposition, as evidenced by the number of adults on each form, feeding damage and eggs laid. The influence of resource fluctuation on the preference was determined by simulating disturbance scenarios of two resource levels. Overall, *C. visenda* does not have preference for either form of *D. unguis-cati*. *H. jureceki* however, appears to be more attracted to the LP form, although this relationship is strongly driven by the high nutrient treatment. There is no preference for either form by the two biological control agents, *C. visenda* and *H. jureceki* under control conditions. The results imply that there is a significant effect of resources (water and nutrient) level on the choice of *H. jureceki* but not *C. visenda*, possibly a result of the different feeding habits by these two biological control agents (see Jermy 1984).

Carvalhotingis visenda (tingid) preference

Tingids were more attracted to the LP form than the SP form under the high nutrient scenario, although the number of tingids reduced after the third day, possibly due to delayed or slow locomotory response of tingids when compared to *H. jureceki*. A field assessment on the establishment of *C. visenda* carried out three years since first release showed that the rate of spread of this agent is slow, estimated at 5.4 m per year (Dhileepan *et al.* 2010). The resource-enemy release hypothesis postulates that high resource plants are likely to be susceptible to insect damage (Blumenthal 2006). According to the theory of fluctuating resource proposed by Davis *et al.* (2000), invasive species are more likely to colonize habitats experiencing fluxes of unused nutrients. Insects are attracted to plants that have high concentrations of foliar nitrogen (McClure 1980). Therefore, the possible explanation for preference of tingids for LP could be that this form accumulates more leaf nitrogen than SP.

Application of additional fertilizers in the HN treatment could have better improved the nutritional status of the LP form, thereby attracting more insects because nitrogen is known to be a limiting nutrient for plant feeding insects (Hosseini *et al.* 2010; Mace and Mills 2015). Few tingids were attracted to both forms when grown under stressful conditions of low resources (LW, LN). However, in a separate experiment in this study, the two forms did not

show any differences in leaf N concentrations when grown under high nutrient conditions (Chapter 6 of this thesis). Thus, this preference of *C. visenda* to LP in high nutrient conditions could be a result of other signals attracting them, perhaps exudates from foliar nectaries or other secondary metabolites (Rosenthal and Berenbaum 2012). However, this is yet to be tested, and therefore, this assertion remains speculative. The seeming preference for LP growing in high nutrient by *C. visenda* did not result in corresponding greater leaf damage on this form.

In the current study, feeding damage by *C. visenda* was estimated by counting the number of leaves showing signs of chlorosis. In general, this study showed that there were fewer signs of leaf damage as a result of *C. visenda* feeding. A possible explanation could be that unlike *H. jureceki* which mine through the leaves causing conspicuous damage, *C. visenda* sucks the sap out of leaves. It has been shown that feeding damage by *C. visenda* is generally low (Williams *et al.* 2008). Therefore, given a short time, as in this study, feeding damage could be minimal. There was also no difference in oviposition preference between the forms at each treatment. The juveniles (neonates) of *C. visenda* are known to mature in 17 ± 1.4 days (Dhileepan *et al.* 2010), thus the time limit of the preference experiments in the current study coupled with use of non-sexed insects could have contributed to this finding.

Hylaeogena jureceki (beetle) preference

The agent *Hylaeogena jureceki* had a significantly higher preference for LP than SP in this study, when considering all treatments. Whereas most treatments only showed marginal preference difference between forms, there was higher preference for LP under the high nutrient scenario (HN) by this agent. Just like *C. visenda*, this agent appear to be attracted by nutritionally enhanced plants with more concentrations of leaf nitrogen (Huberty and Denno 2004). The selective feeding hypothesis posits that leaf miners have preference for leaf tissues high in nitrogen but low in structural tissues (Scheirs *et al.* 2001). This may explain why *H. jureceki* could have had more preference for LP than SP, even though the two forms had similar leaf nitrogen (Chapter 6 of this thesis). Leaf structural compounds like lignin and tannins have been shown to deter leaf miners (Dudt and Shure 1994; Hespenheide 1991), thus acting as defense strategies against herbivory (Caldwell *et al.* 2015). Leaf venation architecture could also affect the choice of insects (Hespenheide 1991). More studies are required to determine the structural traits of LP and SP and specifically test the selective feeding hypothesis to

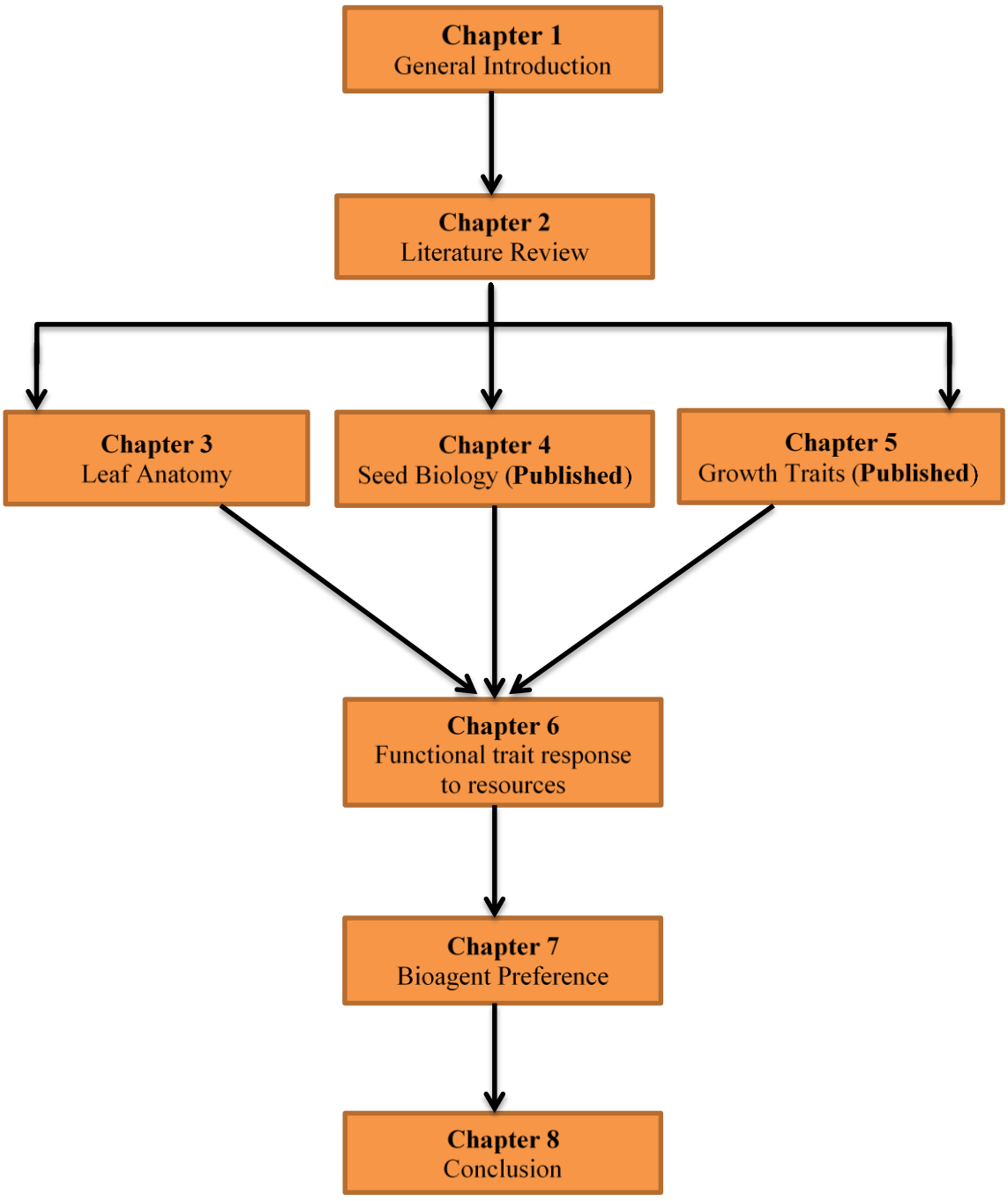
substantiate this position. The preference for LP by *H. jureceki* resulted in higher leaf damage in LP than SP (although the difference was not statistically significant). On the contrary, there was more oviposition of *H. jureceki* on the SP than LP under the control experiments. As the insects were not sexed in this study, and their ages not determined, oviposition results are not informative but show interesting general trends.

Higher preference by *H. jureceki* for the LP has significant implications because it implies that SP could be less attractive to this agent. This scenario could compromise prospects of the biological control program of *D. unguis-cati* in Australia. There have been cases of biological control failures in the past as a result of agent-host mismatch (McFadyen 2003), especially in weed species that have variable forms or varieties (Zalucki *et al.* 2007). For example, *Lantana camara* is a hybrid complex with several morphologically variable forms that have made its biological control programmes challenging globally (Day and Zalucki 2009; Sheppard 1992; Urban *et al.* 2011). The invasive form of lantana is not even native to anywhere because it is a hybrid and that makes potential agents collected from the native range to have lower establishment rate in the new range, which results in biological control failure (Day and Neser 2000). The taxonomic status of the two forms of *D. unguis-cati* is yet to be resolved, but it seems the magnitude of the lantana problem is incomparable to *D. unguis-cati* as it has more than 650 named varieties worldwide (Munir 1996; Nayak *et al.* 2008; Scott *et al.* 2002).

Another invasive species that has shown morphological variation in the introduced range is *Acacia nilotica*, which has up to nine subspecies identified so far (Bargali and Bargali 2009). Hybridizations are known to occur frequently between the subspecies (Ali and Qaiser 1980; Wardill *et al.* 2005), resulting in new forms that could even be more invasive (Culley and Hardiman 2009; Ellstrand and Schierenbeck 2000). Such a scenario jeopardizes the success of a biological control program. Some agents are so host specific to the subspecies to the extent that they will not attack other subspecies. Wardill *et al.* (2005) strongly recommend that biological control efforts dealing with target weed species with taxonomic uncertainty and morphological diversity should ensure accurate genotyping of native populations. This would ensure that the search for potential biological control agents is done in the appropriate locations to avoid agent-host mismatches (Goolsby *et al.* 2006; Keane and Crawley 2002).

Conclusions

Our results show that *C. visenda* does not prefer one form of *D. unguis-cati* over the other. This implies that this biological control agent may be suitable for both LP and SP. On the other hand, *H. jureceki* shows some preference for the LP than SP. Such a preference could cause reduced effectiveness of *H. jureceki* in controlling SP, resulting in uncontrolled spread of this form. However, the two biological control agents tested in this study are reported to be well established in Australia (Dhileepan *et al.* 2010; Snow and Kunjithapatham 2013). The findings from this study contribute to our understanding of the two biological control agent preferences of the two forms of *D. unguis-cati* in Australia. However, this result is not conclusive as the trials were only carried out in the glasshouse under controlled environments. Field studies are needed to assess the preference and impact of all *D. uguis-cati* biological control agents on LP and SP in Australia. A post-release evaluation of the efficacy of biological control agents of *D. unguis-cati* in view of LP and SP would provide valuable information on how effective the agents are and whether there is a need to review the strategy (Briese and Spafford 2003; Morin *et al.* 2009; Sheppard *et al.* 2002).


Chapter 1
General Introduction
Chapter 2
Literature Review
Chapter 3
Leaf Anatomy
Chapter 4
Seed Biology (Published)
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Growth Traits (Published)
Chapter 6
Functional trait response
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Chapter 8: Conclusions and Future directions

8.1 Overall Trends

The overarching objective of the study presented in this thesis was to compare the functional traits of long pod (LP) and short pod (SP) forms of *D. unguis-cati*. Specifically, the study aimed to determine the differences in fitness and performance traits that could help explain why SP appears to be more prevalent than LP, as well as testing whether biological control agents have a similar preference for each form. Overall, research findings from this study contribute to better understanding of the ecological status of the two forms of *D. unguis-cati* in Australia. This is achieved by way of providing a prospectus of fitness traits and showing how each form performs when evaluated against those traits. A grand summary of these traits and how the forms perform is presented in **Table 8.1**.

The study was unique in that it covered a broad spectrum of traits, including (i) dispersal and colonization traits, i.e. germination traits presented in Chapter 4 (Ferreras *et al.* 2015; Mandák 2003); (ii) vegetative traits that affect the ability of plants to compete for resources (Chapters 5 and 6) (Godoy *et al.* 2012; Pyšek and Richardson 2007) and (iii) traits that indicate preference of biological control agents for variable forms of an invasive species (Chapter 7) (Larsson and Ekbom 1995). This study even goes further to evaluate important morpho-anatomical traits and their differences between the two forms (Chapter 3). Most trait-based studies that compare invasive versus non-invasive species rarely include germination traits (e.g. van Kleunen *et al.* 2010b) or anatomical characters (Osunkoya *et al.* 2014). This is despite findings that germination traits have vital demographic consequences for invasive species (Udo *et al.* 2016). Likewise, a recent study by Osunkoya *et al.* (2014) reported significant linkages between performance traits and anatomical characters of invasive versus non-invasive vines in Australia.

The results from this study indicate that SP, the more abundant form of *D. unguis-cati* in Australia, possesses greater performance and fitness capacity of a successful invader than LP, the less abundant form. SP showed greater germination capacity, higher photosynthetic potential and a more coordinated response to changes in environmental resources than LP. This is in agreement with previous studies that have found significant ecophysiological trait

differences between invasive and native plants (Godoy *et al.* 2011). In a meta-analysis comprising 196 non-invasive versus 125 invasive species, van Kleunen *et al.* (2010b) showed that invasive species consistently portrayed greater values for performance traits than non-invasive species. Other pairwise studies involving invasive versus non-invasive species have found that invasive species showed higher phenotypic plasticity (Davidson *et al.* 2011) and higher return on leaf investment (Penuelas *et al.* 2010) than non-invasive species. Findings from investigations described in this thesis indicate that a combination of reproductive, physiological and performance traits confer different ecological strategies that are important in facilitating successful colonization (also see Küster *et al.* 2008).

8.2 Key Findings

There are six take-home messages resulting from this study and they are as follows:

1. From a trait based approach, two forms of a species can have significantly different performance and fitness traits that may potentially enhance the fitness of one and not the other. This study provides some explanation for the differences in abundance levels of the two forms of *D. unguis-cati* and why SP may be the dominant form. In this study, SP was found to have higher values of germination indices, frequency of polyembryony, growth rate, branching capacity and better physiological response to heterogenous environments. Chapters 4, 5 and 6 present this outcome in detail, starting with germination traits, growth traits in low resources and phenotypic response to differences in resource conditions respectively. From the findings, we hypothesised that either SP rapidly evolved an increased competitive ability than LP post-introduction as suggested by the EICA hypothesis (Blossey and Notzold 1995) or possessed this capacity pre-introduction (Firn *et al.* 2011; Schlaepfer *et al.* 2010).

2. SP shows typical germination traits of invasive species, characterised by high germination at a faster rate than LP. Germination experiments described in Chapter 4 of this thesis lasted for 12 weeks and halfway at six weeks, SP had reached maximum germination in most of the temperature regimes. Invasiveness capacity of SP may also have been reinforced by its higher germination plasticity in response to different light and temperature regimes. SP germinated well even at cooler temperature regimes of 10/20 °C whereas LP did not show any signs of germination at this temperatures (Chapter 4). SP also exhibited a higher level of

occurrence of polyembryony, a performance trait that has been reported to increase propagule pressure for some invasive species (Averill *et al.* 2010; Ladd and Cappuccino 2005b). In summary, SP shows traits that enhance capacity for swift colonization of heterogenous environments (Flory and Clay 2009), while LP has a limited germination niche. Slower germination behaviour by LP is a trait associated with more conservative species with less capacity to colonise novel environments (Ferreras *et al.* 2015). If the rate of germination, its plasticity and occurrence of polyembryony are taken into account, the results would give SP higher potential for colonization success, and therefore its greater invasiveness capacity.

Although LP produces more seeds than SP on a per pod basis (Shortus and Dhileepan 2011), the flowering phenology of LP is infrequent and irregular. On the other hand, SP appears to produce flowers multiple times throughout the year especially after rains (personal observation). Some invasive species have shown characteristics of longer flowering periods than non-invasive species (Pyšek and Hulme 2005; Sobrinho *et al.* 2013). Successful colonizers are also known to start reproduction early in their development and SP has shown an indication of this trait because it was the only form that flowered in the glasshouse during this study (**Figure 8.1**). A pairwise assessment of flowering phenology of 227 invasive-native species found significant differences between the two groups (Godoy *et al.* 2009). Invasive species were found to flower earlier and for longer periods than non-invasive species in another study (Pyšek and Richardson 2007). A longer flowering regime by SP could enhance its overall fitness through higher reproductive output resulting in greater colonization potential (Baker 1974; Küster *et al.* 2008; Lake and Leishman 2004; Sobrinho *et al.* 2013).

3. Another important outcome of this study is that under certain conditions, SP exhibits higher physiological and performance traits than LP, implying different carbon economic strategies by the two forms (Jo *et al.* 2015). Variation in leaf traits that facilitate carbon fixation (e.g., SLA, photosynthetic rate and C: N ratio) usually separate fast growing, competitive invasive species from the slow growing non-invasive ones (Leishman *et al.* 2010; Osunkoya *et al.* 2010b). This is in agreement with the leaf economic spectrum (LES) described by Wright *et al.* (2004). From this study, the performance of LP under high light and high nutrient was higher for biomass accumulation (Chapter 6) while SP performed better under low nutrient (Chapter 5 and Chapter 6). According to Funk and Vitousek (2007) some invasive species perform better under low resources and this was the case for SP. SP showed higher physiological performance and resource use efficiency (RUE) than LP under most conditions.

SP also exhibited a higher degree of phenotypic integration of traits, a phenomenon that has also been associated with invasiveness as it enhances robustness in coordinated trait response to change in the environment (Luo *et al.* 2015; Osunkoya *et al.* 2014; Pigliucci 2003). Vines such as LP and SP depend on other plants for support, thus they occupy predominantly low light environments created by overarching tree canopies. Taking into consideration the greater germination capacity (Chapter 4) and higher growth under low light and nutrient resources (Chapters 4-6), it appears that SP has a greater capacity to pre-empt and compete better under these environments that LP. However, the finding that LP performs better under high light and nutrient conditions indicate that this form could be opportunistic, taking advantage of canopy removal and nutrient enhancement to expand its range (Alpert *et al.* 2000; Taylor and Dhileepan 2012; Taylor and Cruzan 2015).

4. While the biological control agent *C. visenda* was equally attracted to both forms of *D. unguis-cati*, *H. jureceki* preferred LP to SP. On the contrary, *H. jureceki* laid more eggs on the SP than LP. The differences uncovered here, though few, could jeopardise management of LP and SP in Australia if they prevail in the field at a larger scale. If indeed LP and SP have different invasiveness potentials as suggested in this thesis, using the same set of biological control agents to manage both forms could be counterproductive. This is even more so as one of the agents, *H. jureceki*, does not prefer the most invasive form, SP. Appropriate control strategies should be focused on those traits that potentially dispose SP to be a more successful invader than LP. For example, agents that attack flowers, seeds and tubers would likely reduce its fitness in combination with the agents currently in use. An effective biological control agent is one that suppress the growth and spread of an invasive species faster than the growth rate of that species (Chaujar 2010). Thus, an understanding of the biology and ecology of an invasive species should be an integral part of the process of designing and implementing a biological control management plan.

5. Some anatomical trait differences observed in this study have taxonomic implications for LP and SP. They include calcium oxalate crystals (Nakata 2003; Rodríguez-Morales *et al.* 2016), different types of epidermal hairs (Juan *et al.* 2000; Osman 2012) and extra-floral or foliar nectaries (Marazzi *et al.* 2013; Nogueira *et al.* 2012; Seibert 1948). This scenario raises questions about the taxonomic placement of LP and SP in Bignoniaceae. The same questions were raised previously by Boyne et al. (2013) upon finding significant variations in leaf types between LP and SP. There have even been suggestions that the two forms may not be the same

species (Scharaschkin, pers. comm). Other anatomical traits could have weed management implications for the two forms of *D. unguis-cati* in Australia. These include different types of trichomes (do Nascimento and Del-Claro 2010; Nogueira *et al.* 2012; Styrsky *et al.* 2006). The LP form was found in this study to have a higher density of hairs than SP (Chapter 3). The occurrence of leaf epidermal hairs is an anti-herbivory strategy by plants (Dalin *et al.* 2008; Metlen *et al.* 2009). Field observations seem to suggest different variations in the hairiness of LP leaves in Australia, ranging from prominently hairy (Boyne *et al.* 2013a) to more glabrous (personal observation).

Foliar nectaries are known to exude sugary nectars that attract ants, and the ants have been associated with anti-herbivory strategies (but see Nogueira *et al.* 2012). During this study, more prominent exudates (and ants visitation) were observed in LP than SP. Ant visitation to feed on extra-floral nectaries has been shown to reduce herbivory in some species (Agrawal and Rutter 1998; do Nascimento and Del-Claro 2010; Oliveira and Freitas 2004). This scenario could have negative impacts on the efficacy of biological control agents on the LP (Styrsky *et al.* 2006). However, more investigations are required to corroborate this assertion.

6. Finally, results of this study confirm the hypothesis that differences between invasive and non-invasive species in functional traits are context dependent (Daehler 2003; Smith and Knapp 2001). For example, total germination percentage was not significantly different between the two forms under high light and warmer temperature regimes (15/25 °C and 20/30 °C), but SP showed significantly higher germination percentage at low light and temperatures. Meanwhile, the rates of germination and occurrence of polyembryony was higher in SP than LP in all conditions (Chapter 4). SP accumulated significantly more biomass than LP when plants were grown under low resources (Chapter 5), but LP had more biomass than SP under high resources (Chapter 6). Phenotypic integration was similar between LP and SP when compared across all light, water and nutrient treatments but differed significantly when high light and high nutrient resources were considered separately (Chapter 6).

Table 8.1 A summary of ANOVA showing the direction of difference for traits measured in this study. The direction of difference is shown in color differences, where black represents the lower value and white represents the higher value for a particular trait in each form. Grey color indicates no significant difference in trait values between the forms. The subscripts represent degrees of freedom. A significant P-value under Trait responses to resources indicates that one form (white) has a significantly greater effect size (treatment response) than the other (black). See Table I for description and units of traits

TRAITS	Summary ANOVA		Direction of difference	
	F statistic	Sig.	LP	SP
LEAF ANATOMY TRAITS				
Epidermal thickness (μm)	$278.2_{1,3}$	**0.0001**		
Palisade mesophyll thickness (μm)	$8.042_{1,3}$	**0.03**		
Spongy mesophyll thickness (μm)	$0.213_{1,3}$	0.7		
Foliar nectaries frequency	$57.348_{1,7}$	**0.001**		
Trichome density$_{adaxial}$	$8.984_{1,3}$	**0.02**		
Trichome density$_{abaxial}$	$4.4_{1,3}$	0.07		
Stomatal density	$27.593_{1,3}$	**0.001**		
Calcium oxalate crystals				
GERMINATION TRAITS				
GRI$_{6weeks}$	174.148	**0.0001**		
GRI$_{12weeks}$	102.664	**0.0001**		
Total germination$_{6weeks}$				
Total germination $_{12weeks}$	82.604	**0.0001**		
Polyembryony frequency	χ^2=71.730	**0.002**		
Polyembryony classes				
GROWTH TRAITS				
Total drymass	$7.455_{2,36}$	**0.005**		
Shoot/root ratio	$4.99_{2,36}$	**0.03**		
Tuber dry mass	$4.923_{2,36}$	**0.03**		
Stem height/length	$20.43_{2,36}$	**0.0001**		
No. of branches	$7.837_{2,36}$	**0.001**		
Apical Dominance Index	$3.191_{2,36}$	**0.09**		
SLA	$3.18_{2,36}$	**0.09**		
LDMC	$0.037_{2,36}$	**0.85**		
Shoot mass ratio	$1.778_{2,36}$	**0.09**		
TRAIT RESPONSE TO RESOURCES				
Total drymass	$8.124_{1,88}$	**0.006**		
Shoot/root ratio	$1.988_{1,88}$	0.2		
No. of tubers	$46.459_{1,88}$	**0.0001**		
Stem diameter	$9.814_{1,88}$	**0.003**		
SLA	$0.454_{1,88}$	0.6		
A_{max}	$4.067_{1,108}$	**0.05**		
A_{mass}	$2.96_{1,31}$	0.09		
ϕPSII	$18.2_{1,108}$	**0.0001**		
WUE	$30.294_{1,108}$	**0.001**		
PNUE	$0.138_{1,31}$	0.71		
Chl.	$58.521_{1,108}$	**0.0001**		
N_{area}	$5.31_{1,31}$	**0.03**		
N_{mass}	$1.162_{1,31}$	0.291		
C	$6.282_{1,31}$	**0.018**		
C:N	$7.289_{1,31}$	**0.01**		
Phenotypic integration	N/A	**N/A**		
AGENT PREFERENCE				
H. juereceki				
Preference	$7.779_{1,50}$	**0.008**		
Feeding damage	$0.673_{1,50}$	0.417		
Oviposition	$8.782_{1,50}$	**0.005**		
C. visenda				
Preference	$0.001_{1,50}$	0.984		

Feeding damage	$1.967_{1,50}$	0.168		
Oviposition	$0.115_{1,50}$	0.737		
Total number of greater values for each form			**6**	**24**

8.3 Future Research Directions

Any research investigation of this magnitude will generate more questions and set the pace for future work and this study is no exception. There are several ways that the current research can be extended to enhance knowledge of the biology, ecology and management of LP and SP.

1. The two forms of *D. unguis-cati* were observed to have a different flowering phenology. It is thus not known whether LP and SP can interbreed as SP was the only form that flowered during this study. There are known weedy species with forms that hybridize, resulting in even more invasive hybrids such as *Lantana camara* (Urban *et al.* 2011) and *Acacia nilotica* (Ali and Qaiser 1980). Interbreeding trials between LP and SP could adopt flower bagging and emasculation methods. This may require treating plants with flowering hormones such as gibberellins to influence plants to produce flowers simultaneously in the glasshouse.

2. Germination experiments described in Chapter 4 have revealed important reproductive differences between LP and SP that have implications for spread. However, only a subset of environmental conditions was investigated, i.e. only light and temperature regimes. An extension of this work involving moisture levels (McLaren and McDonald 2003), seeds buried at different depths (Vivian-Smith and Panetta 2004b) in different conditions. Another outcome of germination experiments was that both forms show polyembryony, with SP showing higher frequency and levels of this phenomenon. The ecological fitness conferred by polyembryony is still largely unexplored in ecological literature. To test the fitness of polyembryony on the two forms of *D. unguis-cati* we suggest assessing growth traits of polyembryonic seedlings in a field study. In this regard, a competition experiment between LP and SP would also be appropriate and provide valuable information on the ecological fitness of the two forms. Closely related to this point, field common garden experiments to evaluate whether LP or SP populations would differ in fitness across simulated habitats would provide a stronger foundation for the comparative trait framework. This point underscores one of the limitations of the current study.

3. This study also showed some indication of preference of *H. jureceki* for LP over SP (Chapter 7). Preference for one form of a variable invasive species by biological control agents could jeopardize biological control efforts. However, this study design only involved a choice experiment. An alternative to this method could be a no-choice experiment where insects are presented to the only one form at a time, followed by determination of leaf damage/plant fitness. We suggest a regular field evaluation of *D. unguis-cati* biological control programmes factoring in the two forms. A typical example was an evaluation of the biological control programme for groundsel bush (Sims-Chilton 2009-10).

Beyond the scope of this thesis, there were some additional aspects that were examined that could set a stage for future works on the two forms of *D. unguis-cati* in Australia. These include the use of an airflow olfactometer to determine whether the biological control agents for *D. unguis-cati* use olfactory cues to choose between LP and SP. I only tested the leaf mining jewel beetle and did not find any indication of olfactory responses, but only tested the agents on control plants. More investigations need to be carried out using plants from different treatments such as those in Chapter 6. If differences in preferences of bio-agents for either form are detected, it could be an indication that the two forms produce different secondary metabolites. This would open another avenue to profile the chemical components of the metabolites using metabolomics methods.

Figure 8.1. Short pod flowering in the glasshouse during studies described in Chapter 6.

8.4 Final Thoughts

Dolichandra unguis-cati was reportedly introduced in Australia as an ornamental plant (Downey and Turnbull 2007), thus the differences in abundance between LP and SP could also have been facilitated by the preferences of gardeners for SP. This is because of the two forms, SP appears more aesthetically appealing with thinner stems (Chapters 5 and 6), manageable foliage (Dhileepan 2012), better climbing habit (Boyne *et al.* 2013a) and showy bright yellow flowers (Shortus and Dhileepan 2011). These characteristics could have endeared SP to be the preferred candidate over LP, resulting in its frequent cultivation and spread in Australia (also see Chaujar 2010). Cox (2004) observed that ornamental plants that later became invasive were attractive to humans (also see Stirton 1978). Thus, this thesis suggests that a dynamic and plastic phenological regime, greater germination capacity and human agency could have facilitated the success of SP in its spread in Australia when compared with LP.

This study is the first to undertake such a comprehensive approach in comparing LP and SP in Australia. The motivation for this study stemmed from the fact that the two forms have significantly different abundance levels in Australia (see Chapter 2 for details). We hypothesised that such a difference in abundance patterns could mean that the two forms have differing invasiveness capabilities. We are aware of the limitations to this hypothesis as it assumed that the two forms have had the same residence time in Australia. Residence time refers to the period a species has stayed in the introduced range (Li *et al.* 2014; Richardson *et al.* 2015). Residence times are important in determining the distribution level of a species in its new range (Pyšek and Jarošík 2005; Wilson *et al.* 2007). This is because different species go through different lag periods before establishing, naturalising and eventually colonizing the novel environment (Carboni *et al.* 2016; Crooks *et al.* 1999; Donaldson *et al.* 2014).

Information on the residence times specific to LP and SP is lacking as earlier records only referred to the species and not the forms (e.g. Downey and Turnbull 2007). There is a general lack of introduction records for most species introduced for ornamental purposes (Harris *et al.* 2007). Herbarium records show approximately a 20 year difference between the first collections of *D. unguis-cati* specimen that appear like LP and SP (Buru *et al.* 2014). However, we know that herbarium collections are highly opportunistic and therefore in this context they are less informative. Inconsistencies have also been previously reported between field surveys of vegetation assemblages and herbaria collections (Garcillán and Ezcurra 2011; Nelson *et al.* 2013; Ponder *et al.* 2001).

It is worth mentioning too that before Osunkoya *et al.* (2009) and Shortus and Dhileepan (2011), the dichotomy between the two forms did not exist in literature. Therefore before recognition of the two forms, herbaria collections only referred to *D. unguis-cati* or the synonym previously used for the species, *Macfadyena unguis-cati*. As a result of lack of introduction history records, this study has had to assume that the two forms were introduced at the same time. If this is so, the inevitable question asked was, "What causes SP to be more successful in its spread than LP?" We believe that this study has provided some answers to this question by providing a detailed prospectus of functional trait values for the two forms. These traits demonstrate that SP possesses more fitness capacities that could have enhanced its success in expanding its range faster than LP. Nevertheless, we advise that our results should be extrapolated with caution as some of the experiments were conducted in controlled

glasshouse environments and thus could not accurately represent the complex dynamics of field conditions.

LP and SP: What are their real taxonomic statuses?

An indirect implication of the vast differences in anatomical and functional traits between LP and SP raise questions about the taxonomic status of LP and SP. Are they the same species? Differences in non-plastic anatomical traits such as types of epidermal hairs, presence/absence of calcium oxalate crystals and shape of the midrib between LP and SP are of considerable taxonomic value. These traits have previously been helpful in resolving taxonomic relations and separating species in Bignoniaceae (Firetti-Leggieri *et al.* 2013; Nogueira *et al.* 2013; Ogundipe and Wujek 2004). This study also uncovered significant differences in epidermal and mesophyll layer thicknesses, stomatal density and foliar nectary distribution patterns between LP and SP (Chapter 3). Although these traits can be plastic, leaf samples used in this study were collected under similar conditions, thus such differences could also be of diagnostic value.

There were also striking differences in germination dynamics and polyembryony occurrence between SP and LP under various light and temperature regimes. Interestingly, a previous study involving *D. unguis-cati* conducted in its native range found that *D. unguis-cati* did not exhibit any polyembryony (Firetti-Leggieri *et al.* 2013). This is in sharp contrast to current polyembryonic findings in SP (40% polyembryony), but is closer to polyembryonic occurrences in LP (<5% polyembryony). Anecdotal evidence and observation of herbarium specimens suggest that the predominant form of *D. unguis-cati* in the native range is similar in appearance to the form being referred to as LP in Australia (Scharaschkin T. personal comm.). Moreover, this study has also uncovered significant differences in growth patterns, tuber development and responses to environmental conditions between LP and SP (Chapters 5 and 6). Previous studies have reported significant differences in fruit morphology, number of seeds per fruit, fruit maturation time (Shortus and Dhileepan 2011) and leaf morphology (Boyne *et al.* 2013a).

In light of the differences shown in the current study and previous findings, we believe it is safe to suggest that LP and SP are different species. However, a comprehensive phylogenetic study of the different forms of the species and other members of the genus

Dolichandra is highly recommended to corroborate this position. From a weed management perspective, the search for biological control agents would benefit greatly from taxonomic resolution of the two forms, ensuring that future surveillances are more focused.

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Appendices

Appendix A: The front page of a published paper below corresponds to Chapter 4 of this thesis.

American Journal of Plant Sciences, 2016, 7, 657-670
Published Online March 2016 in SciRes. http://www.scirp.org/journal/ajps
http://dx.doi.org/10.4236/ajps.2016.73058

Scientific Research Publishing

Germination Biology and Occurrence of Polyembryony in Two Forms of Cats Claw Creeper Vine, *Dolichandra unguis-cati* (Bignoniaceae): Implications for Its Invasiveness and Management

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Received 11 February 2016; accepted 25 March 2016; published 29 March 2016

Appendix B: The front page of a published paper below corresponds to Chapter 5 of this thesis.

NeoBiota 30: 91–109 (2016)
doi: 10.3897/neobiota.30.8495
http://neobiota.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
NeoBiota
Advancing research on alien species and biological invasions

Comparison of growth traits between abundant and uncommon forms of a non-native vine, *Dolichandra unguis-cati* (Bignoniaceae) in Australia

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Academic editor: *M. van Kleunen* | Received 15 March 2016 | Accepted 9 May 2016 | Published 23 June 2016

Citation: Buru JC, Dhileepan K, Osunkoya OO, Firn J (2016) Comparison of growth traits between abundant and uncommon forms of a non-native vine, *Dolichandra unguis-cati* (Bignoniaceae) in Australia. In: Daehler CC, van Kleunen M, Pyšek P, Richardson DM (Eds) Proceedings of 13th International EMAPi conference, Waikoloa, Hawaii. NeoBiota 30: 91–109. doi: 10.3897/neobiota.30.8495

Appendix C: Paper published in the proceedings of the 19th Australasian Weeds Conference, 2014. This paper is part of chapter 4. The full paper can be accessed at http://eprints.qut.edu.au/78887/ .

Nineteenth Australasian Weeds Conference

Seed germination may explain differences in invasiveness and prevalence: a case study using cat's claw creeper (*Dolichandra unguis-cati*)

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Summary High germination rates and rapid germination behaviour in response to different environmental cues are traits that may be associated with invasiveness. Cat's claw creeper (*Dolichandra unguis-cati* (L.) Lohmann (syn. *Macfadyena unguis-cati* (L.) Gentry), a Weed of National Significance has two forms, a long-pod (LP) form and a short-pod (SP) from. The LP form occurs in only a few localities in south-east Queensland while the SP form is widely distributed in Queensland (Qld) and New South Wales (NSW). The aims of this investigation were: to evaluate whether there are significant differences in germination traits between the two forms of cat's claw creeper; and if there are any significant differences, to find out whether the

recruitment of a species in a new environment (Ranal and Santana 2006). High versatility in germination characteristics can be selected for because the evolutionary success of any organism is directly proportional to the number of individuals in existence and the range of environmental conditions under which they can survive and proliferate in (Baker 1974).

Cat's claw creeper is a Weed of National Significance (Dhileepan *et al.* 2013). It is native to the Greater and Lesser Antilles, Mexico, South and Central America to Argentina, including Trinidad and Tobago (Gentry 1983). Cat's claw creeper was introduced to Australia as an ornamental plant and naturalised in Queensland by the 1950s (Downey and

Appendix D: Conference Abstracts

Conference Abstract 1

Conference: Society for Conservation Biology 4th Oceania Congress; 5-8 July 2016, Brisbane, Australia

Title: Understanding weed ecophysiology for control purposes: a synopsis

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Cat's claw creeper is a Weed of National Significance which has devastating effects in Australian riparian ecosystems. In Australia, this species has two forms, a long-pod (LP) form and a short-pod (SP) form, with different prevalence rates. Two distinct forms may compromise biocontrol strategies for this invasive species. The aims of this investigation were: to understand the ecophysiology of the two forms and test for preference of biocontrol agents to either form. Seeds of the two forms were germinated under various light and temperature regimes. Seedlings were divided into different light, nutrient and water treatments for growth related traits. Some plants were used to test preference of two biological agents on the two forms. Short pod depicted higher rates of germinability and superior growth related traits than long pod. Assuming that the two forms were introduced in Australia at around the same period, these results could explain why SP is widely distributed (and therefore more invasive) in Qld and NSW while LP is only confined to a few localities in south-east Queensland. These results infer that different management strategies should be adopted in controlling the two forms in Australia.

Conference Abstract 2

Conference: 13th International Conference on Ecology and Management of Alien Plant Invasions (EMAPi); 20-24 September 2015, Hawaii USA

Title: Tuber development and growth rates of two varieties of an invasive liana, *Dolichandra unguis-cati* in Australia

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Cat's claw creeper (*Dolichandra unguis-cati* (L.) Lohmann (syn. *Macfadyena unguis-cati* (L.) Gentry), a Weed of National Significance has two forms, a long-pod (LP) form and a short-pod (SP) from. The LP form occurs in only a few localities in southeast Queensland while the SP form is widely distributed in Queensland and New South Wales. The aims of this investigation were: to evaluate whether there are significant differences in tuber development and root/shoot ratio between the two forms of cat's claw creeper; and if there are any significant differences, to find out whether the differences in resource allocation can be related to prevalence and invasiveness levels for the two forms. Long pod and short pod seeds collected in 2013 from various localities in Qld were germinated in growth chambers. Seedlings were then grown in a greenhouse for 18 months with regular watering but no additional nutrients. Harvesting of plants was done at 5 months, 10 months and 18 months respectively. Tuber size, root dry mass and shoot dry mass etc were measured for both forms of cat's claw creeper. SP exhibited significantly higher total number of tubers per plant and tuber size than LP. Root/shoot ratio was also significantly different between the two forms and the SP exhibited a high level of branching than LP. Assuming that the two forms were introduced in Australia at around the same period, these results could explain why SP is widely distributed (and therefore more invasive) in Qld and NSW while LP is only confined to a few localities in southeast Qld. These results infer that different management strategies should be adopted in controlling the two forms in Australia.

Conference Abstract 3

Conference: International Student Conference on Conservation Science; 19-25 January 2015, Brisbane, Australia

Title: The role of plant anatomy in invasion ecology

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According to the Convention on Biological Diversity (CBD), exotic invasive species are the second largest cause of biodiversity loss globally. For a long time invasion biologists have embarked on a search for traits that could confer invasiveness on introduced species. Several hypotheses have been proposed to explain invasiveness. Higher ecophysiological performance by some species account for invasiveness, however, most ecological studies fail to clearly draw linkages between leaf anatomy and ecophysiological performance. The leaf is the site for gaseous exchange and primary production via photosynthesis. Micro-techniques can be used to study the internal anatomy of invasive vs non-invasive species and then relate with ecophysiological performance. These include leaf impression, free hand sectioning, paraffin embedding and scanning electron microscopy (SEM). This presentation will discuss leaf anatomical and micro-morphological traits of two forms of *Dolichandra unguis-cati*, a Weed of National Significance in Australia. These two forms (‘long pod and short pod’) have different levels of invasiveness in Australia and therefore an ideal system for such a comparative study.

Conference Abstract 4

Conference: 19th Australasian Weeds Conference; 1-4 September 2014, Hobart Australia

Title: Seed germination may explain differences in invasiveness and prevalence: a case study using cat's claw creeper.

Buru, J.C. (1), Osunkoya, O (2), Dhileepan, K (2) and T. Scharaschkin (1)

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Cat's claw creeper, *Dolichandra unguis-cati* (Bignoniaceae) is a major environmental weed in southeast Queensland (SEQ) and New South Wales (NSW). Two forms of this weed, with distinct leaf and fruit morphology, are reported to occur in SEQ. The long pod (LP) form occurs in only a few localities in SEQ while the short pod (SP) is widely distributed in Queensland and NSW. The aim of this investigation was to evaluate the germination dynamics of cat's claw creeper by comparing LP and SP. Seeds of both LP and SP were collected from localities where the two co-occur and from other locations where individual forms are found. Seeds were germinated in growth chambers under 10/20^{O}C, 15/25^{O}C, 20/30^{O}C and in ambient conditions at room temperature. Two light conditions were imposed, total darkness and a 12-hour photo-period for each temperature regime. Germination was monitored over a period of 12 weeks. For all the treatments, SP exhibited significantly higher total germination percentage and rates of germination than LP. At 10/20^{O}C, LP seeds did not germinate but SP showed lower germination rates. SP exhibited about 30% poly-embryony as opposed to less than 5% exhibited by LP. These results could explain why SP is more widely distributed (more invasive) in Queensland and NSW while LP is only confined to a few localities in SEQ.

Conference Abstract 5

Conference: Australasian Systematic Botany Society (ASBS) Conference; December 2013, Sydney Australia

Title: Anatomical and micro-morphological variation in the leaves of *Dolichandra unguis-cati* (Bignoniaceae)

***Buru, J.C.** and T. Scharaschkin

School of Earth, Environment and Biological Sciences, Faculty of Science and Engineering, Queensland University of Technology, P.O.Box 2435, Brisbane 4001, Queensland, Australia.

Cat's claw creeper, *Dolichandra unguis-cati* (Bignoniaceae) is a major environmental weed in southeast Queensland and New South Wales (NSW). Two forms of this weed, with distinct leaf morphology, are reported to occur in Queensland. Due to the difference in the length of the fruit, these have been called long pod (LP) and short pod (SP). Leaves of both SP and LP were collected from localities where the two co-occur, but only if the form could be clearly ascertained by the presence of mature fruits. The leaves were fixed in FAA in the field and embedded in paraffin wax. The leaves were sectioned using a rotary microtome and stained with toluidine blue, Safranin and fast green. Anatomical and micro-morphological attributes of the two forms of *D. unguis-cati* compared include thickness of lower and upper epidermis, thickness of the palisade mesophyll, thickness of the upper and lower cuticle, stomatal density, presence or absence of hairs and glands on the surface of the leaf. These leaf traits are important since they may influence the functionality of the plant and susceptibility to attack by potential bio-control agents. The outcome of this study will provide baseline data that will influence the taxonomic resolution of the two forms.

***I was awarded the Bob Anderson Memorial Award at this conference**.

Appendix E: Awards and Grants

1. **Bob Anderson Memorial Award (ASBS Conference 2013).**
2. **Student Travel Award: Weed Society of Queensland (WSQ)** – The money from this award was used to travel to the 19th Australasian Weed Conference – valued at AUD 1,711.00.
3. **Student Travel Award: Council of Australasian Weed Societies (CAWS)** – This was used to cover some costs while attending the 13th EMAPi Conference- valued at AUD 1,000.00 (Please see the report at http://caws.org.au/winners/2015_Buru_report.pdf).
4. **Earth, Environment and Biological Sciences (QUT) Early Career Travel Grant** – The grant was used to cover costs while attending the 13th EMAPi Conference - valued at AUD 2,500.00.

Appendix J: Membership of Professional Societies

1. Weed Society of Queensland (WSQ).
2. Council of Australasian Weed Societies (CAWS).
3. Australasian Systematic Botany Society (ASBS).
4. Ecological Society of Australia (ESA).
5. Society for Conservation Biology (SCB).
6. Associate Fellow of the UK Higher Education Academy (HEA): awarded fellowship in 2015.

Appendix K: Continuous Professional Education (CPE) completed during my PhD

February – July 2015:	**Teaching Advantage Course (QUT)**: This course involved Unit/course design and coordination, Effective assessment and feedback, Principles of effective learning, teaching philosophies and portfolios, managing classrooms, pathways to academic careers etc.
January 2015:	**Introduction to R and GIS**: This workshop was part of the International Student Conference on Conservation Science (SCCS) and it included introduction to the R statistical package, introduction to geo-spatial analysis in R (ArcGIS and qgis), Species Distribution Models using Maxent, and Grant Application.
October 2014:	**Leadership and Communication** (Australian Technology Network of Universities) – Learning Employment Aptitudes Program: This five week course involved effective leadership and communication skills, the interrelationship between communication, mentorship, team participation, interpersonal skills and leadership development.
June 2014:	**Project Management** (Australian Technology Network of Universities) – Learning Employment Aptitudes Program: This 5 week course involved understanding the nature of projects, models for effective project management, stakeholder management in projects, processes of project management, project planning and integration.
April 2014:	**Plant Anatomy for Scientists (QUT)**: This three day course was rigorous and dense, covering the rudiments of plant anatomy. WE learnt about plant body parts, plant cell structure, tissues and cell diversity, the anatomy of stems, roots and shoots, meristems, primary and secondary growth lectures and practical sessions to learn free hand sectioning, leaf impressions and slide making, cell identification and staining.